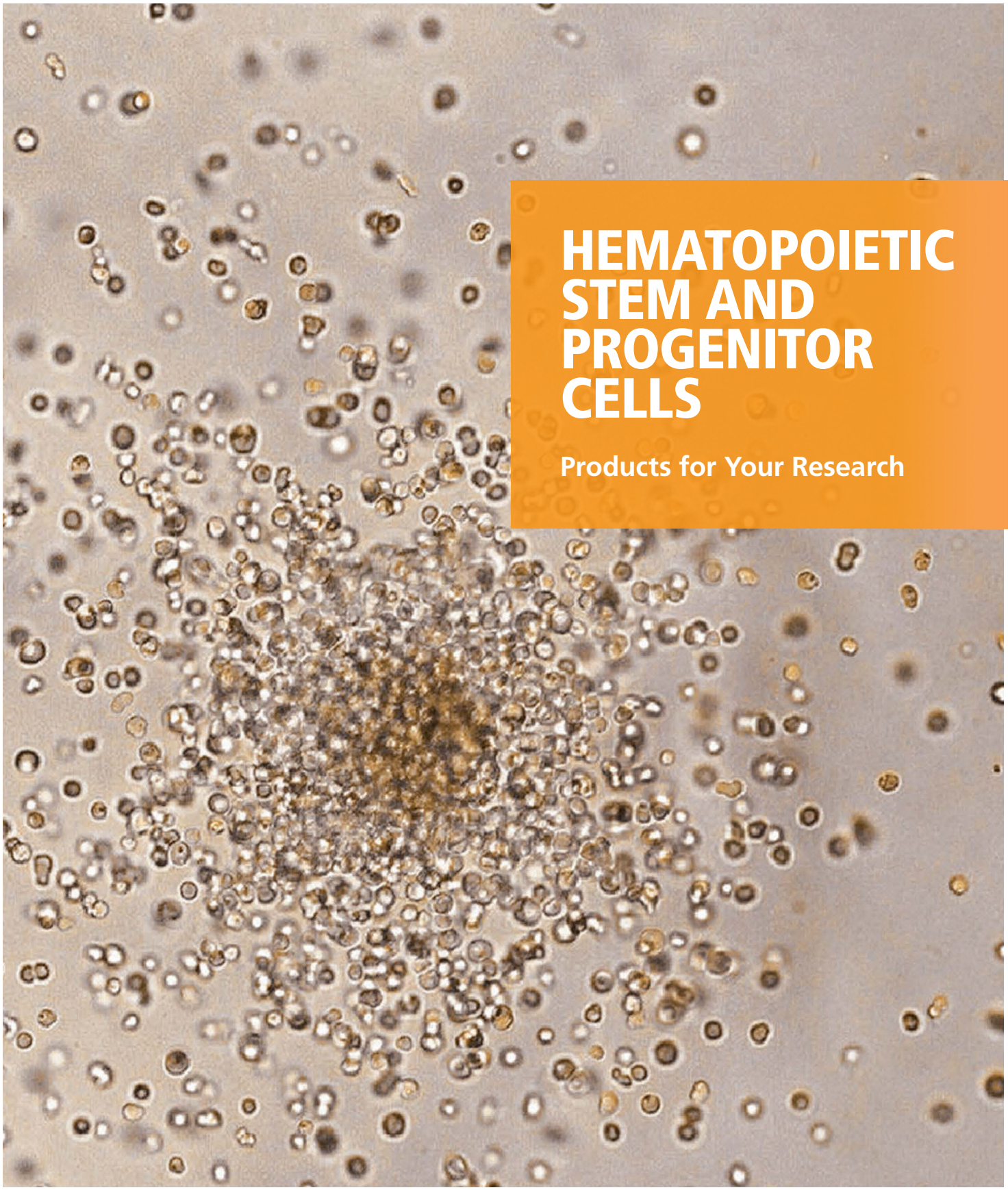

HEMATOPOIETIC STEM AND PROGENITOR CELLS

Products for Your Research

STEMCELL™ TECHNOLOGIES

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Your Ideas. Our Tools.

Products for Every Step of Your Hematopoietic Stem and Progenitor Cell Research

STEMCELL Technologies is the world leader in developing tools for hematology ranging from fundamental research to cell therapy development. Our portfolio includes a comprehensive range of products for cell sourcing and isolation, expansion and differentiation, and analysis of hematopoietic stem and progenitor cells (HSPCs). To help ensure standardization throughout your HSPC research, use STEMCELL products from the beginning to the end of your workflow.

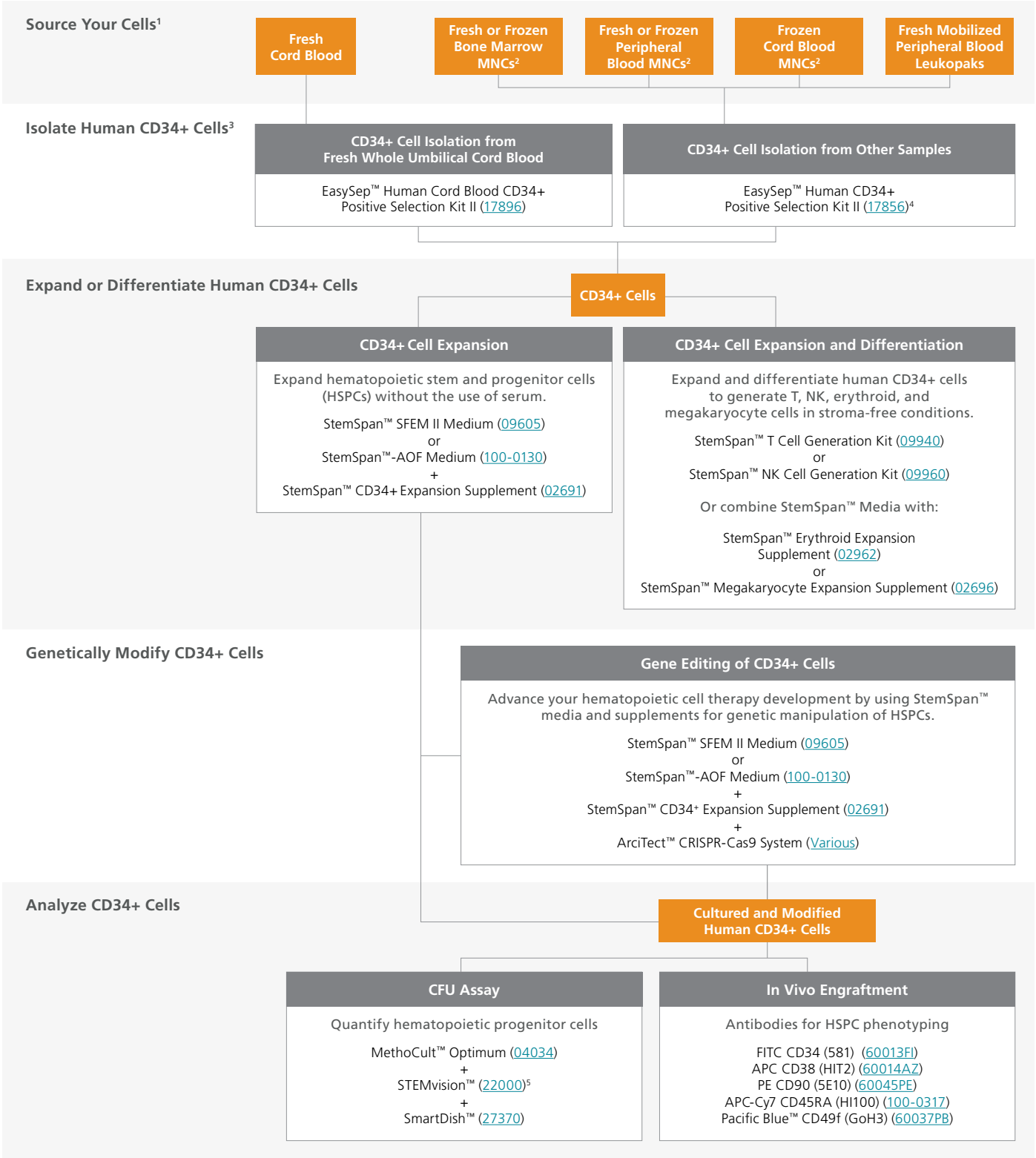
STEMCELL Products for Every Step of Your HSPC Research

Cell Sourcing & Isolation	Expansion & Differentiation	Analysis
Human Primary Hematopoietic Cells	StemSpan™ Media & Kits	MyeloCult™ Media
Cell Isolation Products & Platforms	StemSpan™ Supplements	MethoCult™ Media
EasySep™	STEMdiff™ Kits for Differentiation of hPSCs[1] to Hematopoietic Progenitor Cells	STEMvision™ Instrument
RoboSep™	Recombinant Cytokines	MegaCult™ Media
SepMate™	Small Molecules	Antibodies and ELISA Kits
RosetteSep™		Proficiency Testing Programs
HetaSep™		Contract Assay Services
Cryopreservation Media		

1. *hPSCs: human pluripotent stem cells*

Visit **www.stemcell.com/HSPCworkflow** for a full listing of products for your HSPC research.

Hematopoietic Cell Therapy Development Workflow



1. *Certain products are only available in select territories. Please contact your regional Sales representative or Product and Scientific Support at techsupport@stemcell.com for further information.*
2. *MNCs: Mononuclear cells*
3. *Explore the Human CD34+ Cell Isolation Product Selection infographic for a complete list of recommendations.*
4. *For large-scale isolation of human CD34+ cells from whole blood and normal or mobilized leukopaks, see the large-format ($1x10^{10}$ cells) kit (Catalog # 100-1569).*
5. *Additional validation for specific application may be required.*

Human Primary Hematopoietic Cells

It All Starts with the Right Cells

Cell Sourcing & Isolation | Expansion & Differentiation | Analysis

Starting with the right primary cells builds a strong foundation for your experiments and is the first step toward success in your research. Choose from a wide range of fresh and cryopreserved human primary cells for your downstream applications.*

STEMCELL's cryopreserved hematopoietic cells are isolated from human cord blood, bone marrow, peripheral blood, and mobilized peripheral blood leukopaks. Whole bone marrow, whole peripheral blood, and leukapheresis (leukopak) preparations are also available for users requiring fresh, unprocessed tissue. All products are verified for purity and viability—ensuring reproducibility across multiple experiments.

Visit **www.stemcell.com/primarycells** for a complete list of mononuclear cells, isolated subsets, plasma, serum, and unprocessed tissues.

Donor Criteria

All human primary cell products are ethically sourced using consent forms and protocols approved by either an Institutional Review Board, the U.S. Food and Drug Administration, the U.S. Department of Health and Human Services, and/or an equivalent regulatory authority. Donations performed in the United States comply with applicable federal, state, and local laws, regulations, and guidance. Cells sourced in the United Kingdom are collected using protocols and informed consent forms (ICFs) approved by the National Health Service (and Health Research Authority) Research Ethics Committee (REC) or equivalent agency. Donors are prescreened for general health and viral status, including HIV-1, HIV-2, hepatitis B, and hepatitis C**. Additional screening or analysis is available upon request. Our Quality Assurance, Quality Control, and Regulatory Affairs departments are ready to assist you with any necessary documentation to meet specific institutional requirements.

Resource

Explore Frequently Asked Questions on Primary Cells

www.stemcell.com/primarycellsfaqs

Why Use Human Primary Cells from STEMCELL Technologies?

EFFICIENT. Reduce time spent collecting and culturing primary cells.

CONVENIENT. Reserve large numbers of cryopreserved cells and start experiments on your schedule with cells you've already tested.

CUSTOMIZABLE. Request custom products for non-standard cell types or collections with specific requirements.

PHYSIOLOGICALLY RELEVANT. Choose cells that are more physiologically representative of cells in vivo.

ETHICALLY SOURCED. Access donor samples collected using regulatory authority-approved consent forms and protocols.

Figure 1. Fresh Whole Bone Marrow

Whole Bone Marrow (Catalog #70502) is collected using heparin as the anticoagulant and supplied in a 100 mL bottle.

Certain fresh and cryopreserved products are only available in select territories. Please contact your regional Sales representative or Product and Scientific Support at **techsupport@stemcell.com for further information.*

*** Leukopak, Whole Blood, and Bone Marrow (LP, WB, and BM) Donor Screening: Donors are screened for HIV-1, HIV-2, hepatitis B, and hepatitis C. Donors in the U.K. are also screened for HTLV I/II and syphilis. For fresh mobilized leukopaks donors are screened for HIV-1, HIV-2, hepatitis B, hepatitis C, HTLV-I/-II, syphilis, and WNV. Cryopreserved and fresh LP, WB, and BM: If the donor has been screened within 90 days prior to donation and the results are negative, the product will be shipped with the negative test result and date of the most recent viral testing on the Certificate of Analysis (CoA). If the donor has not been screened within 90 days prior to collection, a test sample will be taken at the time of collection (with the exception of cancer patient donors) and the product will be shipped before the screening results are available. In the event that a test result is positive, the customer will be contacted as soon as possible (usually within 2 - 4 business days from the time of shipment). Fresh Cord Blood (CB) Donor Screening: Testing for HIV-1, HIV-2, hepatitis B, and hepatitis C is performed on a maternal blood sample and/or on a sample of the donated CB. Cryopreserved CB: Products with negative test results from donor screening are shipped with the CoA.*

Product Listing

Fresh Human Bone Marrow Products*

Description	Anticoagulant	Quantity	Catalog #
Whole Bone Marrow	Heparin	> 25 mL	70502.2
		> 50 mL	70502.1
		> 100 mL	70502

Leukopaks*

Description	Anticoagulant	Quantity	Catalog #
Fresh Peripheral Blood Leukopak[1]	ACDA[2]	Tenth Size	200-0092
		Quarter Size	70500.2
		Half Size	70500.1
		Full Size	70500
Frozen Peripheral Blood Leukopak	ACDA[2]	Tenth Size	200-0470
		Quarter Size	200-0132
		Half Size	200-0131
		Full Size	200-0130

Cryopreserved Human Umbilical Cord Blood Products*

Description	Quantity	Catalog #
Mononuclear Cells	15 million cells	70007.1
	50 million cells	70007.2
	150 million cells	70007
CD34+ Cells (Mixed Donor)	0.2 million cells	70008.1
	0.5 million cells	70008.3
	1 million cells	70008
CD34+ Cells (Single Donor)[3]	0.2 million cells	70008.2
	0.5 million cells	70008.4
	1 million cells	70008.5

Cryopreserved Human Bone Marrow Products*

Description	Quantity	Catalog #
Mononuclear Cells	5 million cells	70001.1
	15 million cells	70001.2
	25 million cells	70001
	50 million cells	70001.3
	100 million cells	70001.4
CD34+ Cells	0.1 million cells	70002.1
	0.3 million cells	70002.2
	0.5 million cells	70002.3
	1 million cells	70002
	2 million cells	70002.4
	5 million cells	70002.5
CD36+ Cells[4]	1 million cells	70003

Cryopreserved Human Peripheral Blood Cells*

Description	Quantity	Catalog #
Mononuclear Cells	15 million cells	70025.1
	25 million cells	70025.2
	50 million cells	70025.3
	100 million cells	70025

Cryopreserved Mobilized Human Peripheral Blood Products*

Description	Quantity	Catalog #
G-CSF Mobilized Mononuclear Cells	5 million cells	70049.4
	15 million cells	70049.2
	25 million cells	70049.3
	50 million cells	70049.1
	100 million cells	70049
G-CSF Mobilized CD34+ Cells	0.2 million cells	70060.2
	1 million cells	70060.1
	5 million cells	70060
	10 million cells	70060.3
	20 million cells	70060.4
G-CSF and Plerixafor Mobilized Mononuclear Cells	5 million cells	70072.4
	15 million cells	70072.2
	25 million cells	70072.3
	50 million cells	70072.1
	100 million cells	70072
G-CSF and Plerixafor Mobilized CD34+ Cells	0.2 million cells	70073.2
	1 million cells	70073.1
	5 million cells	70073
	10 million cells	70073.3
	20 million cells	70073.4
Plerixafor Mobilized Mononuclear Cells	5 million cells	70074.4
	15 million cells	70074.2
	25 million cells	70074.3
	50 million cells	70074.1
	100 million cells	70074
Plerixafor Mobilized CD34+ Cells	0.2 million cells	70075.2
	1 million cells	70075.1
	5 million cells	70075
	10 million cells	70075.3
	20 million cells	70075.4

For a complete listing of fresh and cryopreserved products, visit **www.stemcell.com/human-primary-cell-products**.

** Certain fresh and cryopreserved products are only available in select territories. Please contact your Sales representative or Product and Scientific Support at **techsupport@stemcell.com** for further information.*

1. *A full size leukopak typically contains 1.1 ± 0.3 x 10^{10} cells and has a volume of approximately 120 mL.*
2. *ACDA: acid citrate dextrose solution A.*
3. *Additional sizes such as 0.6 million cells, 0.7 million cells, and 0.8 million cells are also available for umbilical cord blood-sourced CD34+ cells (Single Donor).*
4. *Cultured cell product.*

Mobilized Leukopaks[1,2]

Description	Anticoagulant	Quantity	Apheresis	Catalog #
Fresh Human Mobilized Peripheral Blood Leukopak, G-CSF	ACDA[3]	1 bag	First Collection (Day 5)	200-0602
			Second Collection (Day 6)	200-0603
		2 bags	Both Collections (Day 5 and 6)	100-1101 (200-0602 & 200-0603)
Fresh Human Mobilized Peripheral Blood Leukopak, Plerixafor	ACDA[3]	1 bag	First Collection	200-0604
Fresh Human Mobilized Peripheral Blood Leukopak, G-CSF and Plerixafor	ACDA[3]	1 bag	First Collection (Day 5)	200-0607
			Second Collection (Day 6)	200-0608
		2 bags	Both Collections (Day 5 and 6)	100-1103 (200-0607 & 200-0608)

[1] Certain cryopreserved products are only available in select territories. Please contact your Sales representative or Product and Scientific Support at ***techsupport@stemcell.com*** *for further information.*

[2] The cytokine G-CSF or filgrastim (Neupogen®) is the most commonly used mobilizing agent and is used as a gold standard in the clinic. The bicyclam plerixafor (Mozobil®) is a rapid-acting mobilization agent. The combination of both mobilization agents works synergistically, increasing the CD34+ mobilization compared to single-agent mobilization.

[3] ACDA: acid citrate dextrose solution A.

Mobilizing Agent	Mobilizing Regimen	Mobilization and Collection Schedule						Quantity	Apheresis	Catalog #
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6			
Granulocyte Colony-Stimulating Factor (G-CSF)	5-Day					First Collection	Second Collection	1 Bag	First Collection (Day 5)	200-0602
									Second Collection (Day 6)	200-0603
								2 Bags	Both Collections (Day 5 and 6)	100-1101 (200-0602 & 200-0603)
Plerixafor	1-Day	First Collection						1 Bag	First Collection	200-0604
G-CSF + Plerixafor (Combo)	5-Day					First Collection	Second Collection	1 Bag	First Collection (Day 5)	200-0607
									Second Collection (Day 6)	200-0608
								2 Bags	Both Collections (Day 5 and 6)	100-1103 (200-0607 & 200-0608)

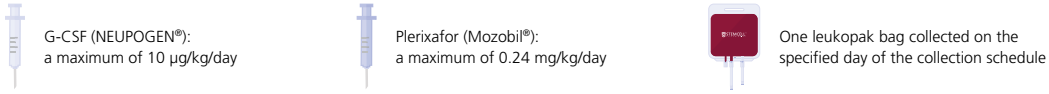


Figure 2. Mobilized Leukopak Selection Guide

Normal donors are mobilized with specified doses of G-CSF, plerixafor, or both mobilizing agents, based on the regimen shown above, prior to collection. The time interval between injection and apheresis can be found listed in the Certificate of Analysis.

Cell Isolation Products & Platforms

Fast and Easy Hematopoietic Stem and Progenitor Cell Isolation

We have combined our years of technical expertise in hematopoietic stem cell research with our powerful cell separation systems to develop a wide range of optimized hematopoietic stem and progenitor cell (HSPC) isolation products. Our innovative cell separation platforms provide fast, easy, and effective methods for isolating HSPCs with high purity and recovery.

EasySep™

The Easy Choice for Simple Cell Isolation

Isolating HSPCs can be a challenge due to their low frequency in tissues. EasySep™ column-free immunomagnetic cell isolation technology is the ideal method for the gentle, high-purity isolation of HSPCs and can be used with a variety of sources, including cord blood, whole blood, and bone marrow.

Using EasySep™, HSPCs can be easily and quickly isolated based on lineage-specific markers, such as expression of CD34 (for human cells), or c-KIT and SCA1 (for mouse cells). EasySep™ can be used either manually or with RoboSep™, the fully automated cell separator.

Why Use EasySep™ to Isolate Cells?

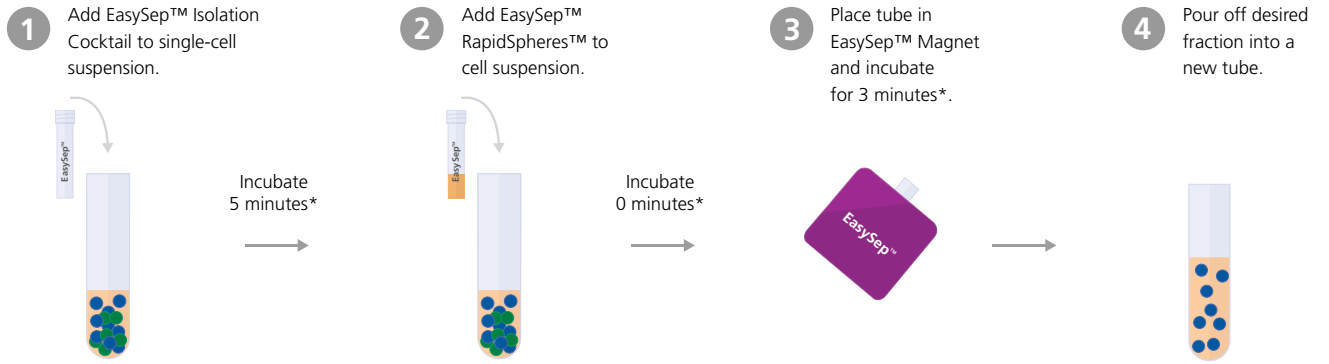
EASY & EFFICIENT. Isolate cells in as little as 8 minutes with a simple pour.

HIGH PURITY & RECOVERY. Achieve up to 99% cell purities with high recoveries.

COLUMN-FREE. Obtain viable, functional cells without the need for columns and washes.

VERSATILE. Isolate cells from virtually any sample source, including whole blood and leukopaks.

PROVEN. Choose a method widely used in published research for over 20 years to support diverse downstream applications.



**Times are typical for next-generation negative selection kits. Time for each kit will vary depending on the exact isolation protocol and magnet used. No particle incubation step is required for next-generation negative selection protocols.*

Figure 3. Typical EasySep™ Human Cell Isolation Protocol

RoboSep™

Fully Automated Cell Isolation System

Automate your cell processing and isolations using RoboSep™ cell isolation instruments. The RoboSep™-S and RoboSep™-16 instruments are true walk-away automated cell separation systems that retain the speed and simplicity of EasySep™ cell separation technology, with as little as 5 minutes of hands-on time. The column-free system minimizes sample handling and eliminates the risk of cross-contamination between samples. You can isolate cells from up to 16 samples at once for flow cytometry, functional studies, or other downstream applications.

RoboSep™-S

RoboSep™-16

SepMate™

Hassle-Free PBMC Isolation

SepMate™ is a specialized tube for fast and easy peripheral blood mononuclear cell (PBMC) isolation in just 15 minutes. The SepMate™ tube contains a unique insert that prevents the density gradient medium (e.g. Lymphoprep™) and blood sample from mixing. The density gradient medium is pipetted through a central hole in the insert, and the sample is rapidly pipetted or poured on top of the insert. This eliminates the need to carefully layer the sample directly onto the density gradient medium, an otherwise time-consuming and highly laborious step. Only 10 minutes of centrifugation are required, and this step can be carried out with the brake on, further reducing the total time necessary for separation. After centrifugation, plasma and PBMCs are simply poured into a new tube.

SepMate™ can be used on its own to isolate PBMCs in 15 minutes or combined with RosetteSep™ to enrich specific cell subsets, including CD34+ cells, directly from whole blood in 25 minutes. SepMate™ is available in 15 mL and 50 mL sizes for isolating individual samples of 0.5 - 17 mL in volume.

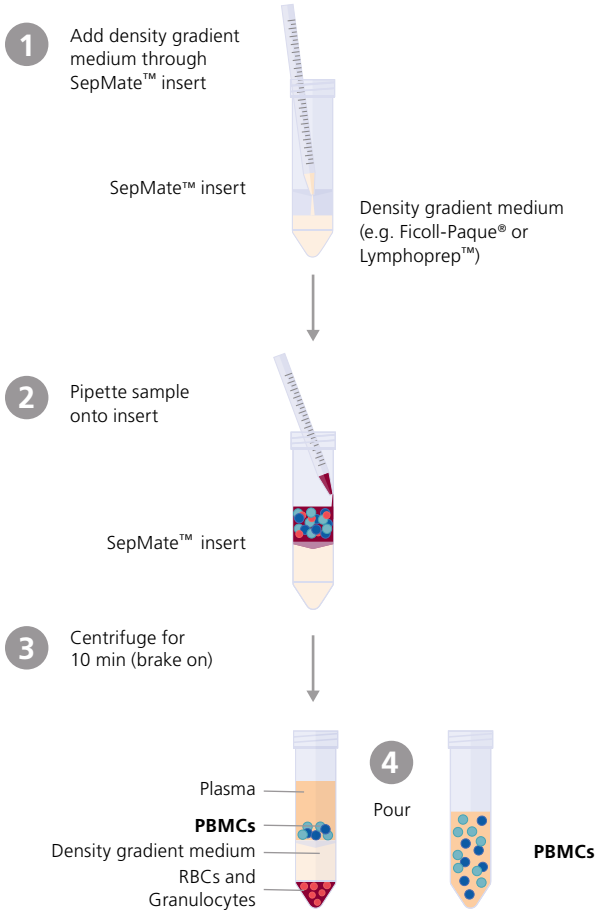


Figure 4. Typical SepMate™ Protocol

Why Use SepMate™?

EASY. Avoid slow and laborious sample layering over the density gradient meduim.

FAST. Centrifuge for just 10 minutes with the brake on and simply pour off PBMCs into a new tube.

CONSISTENT. Eliminate errors and minimize variability between users.

VERSATILE. Combine with RosetteSep™ to isolate purified cell subsets from whole blood in 25 minutes.

Product Listing

Product Name	Catalog #	Blood Volume Processed	Unit Size
SepMate™-15 (IVD[1])	85415 85420	0.5 - 5 mL	100 tubes 500 tubes
SepMate™-15 (RUO[2])	86415 86420		100 tubes 500 tubes
SepMate™-50 (IVD[1])	85450 85460	4 - 17 mL	100 tubes 500 tubes
SepMate™-50 (RUO[2])	86450 86460		100 tubes 500 tubes
Product Name	**Catalog #**	**Density**	**Unit Size**
Lymphoprep™[4]	18060 18061 07811 07861	1.077 g/mL[3]	250 mL 500 mL 4 x 250 mL 6 x 500 mL

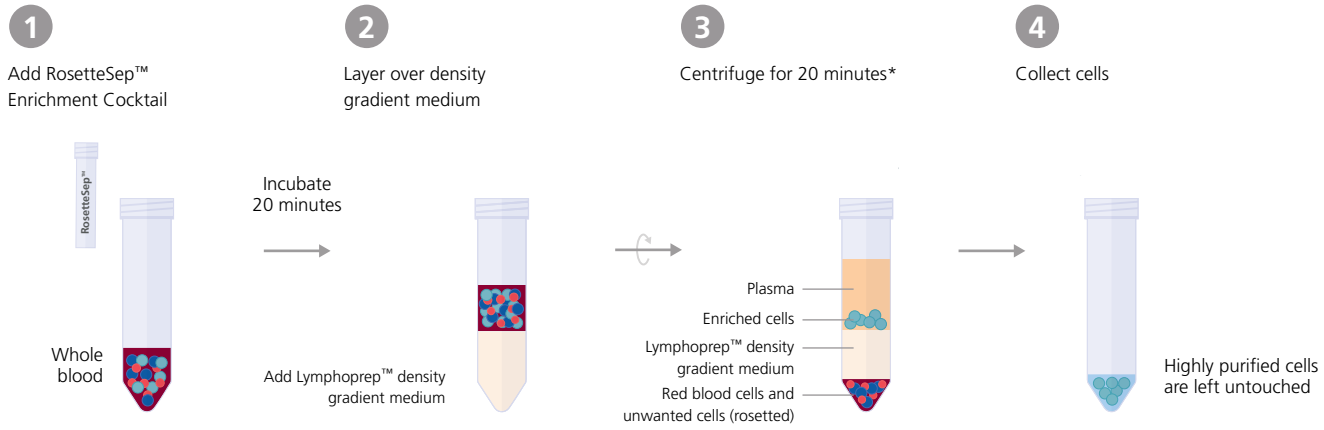
1. *SepMate™ is available as an in vitro diagnostic device for the isolation of mononuclear cells from human whole blood or bone marrow by density gradient centrifugation in Canada, the United States, Europe, and Australia. This product is also available in China where it is considered a non-medical device by the China Food and Drug Administration (CFDA) and should therefore be used as general laboratory equipment.*
2. *SepMate™ RUO is available in other regions for research use only.*
3. *Lymphoprep™ has the same density as Ficoll-Paque® and can be substituted for Ficoll-Paque® without any need to change your existing protocols.*
4. *Lymphoprep™ is for Research Use Only (RUO).*

RosetteSep™

Unique Immunodensity Cell Isolation

RosetteSep™ is a fast and easy immunodensity procedure for the isolation of untouched cells directly from whole blood. By crosslinking unwanted cells to red blood cells (RBCs) present in the sample, RosetteSep™ eliminates the need for a separate magnetic separation step because cells are purified during standard density gradient centrifugation. This approach significantly reduces sample handling time and maximizes convenience.

Typical RosetteSep™ Protocol



**Use SepMate™ to reduce centrifugation time to 10 minutes with brake on.*

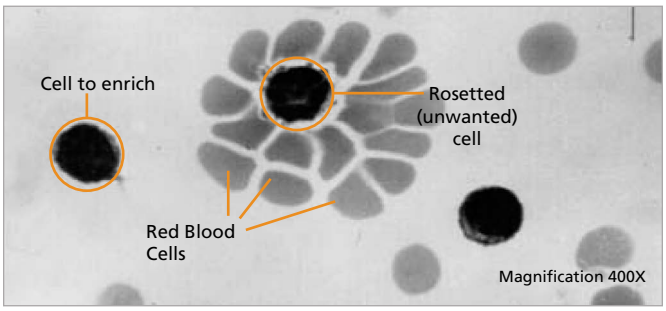


Figure 5. Micrograph of a Blood Sample After Addition of the RosetteSep™ Cocktail and Prior to Density Gradient Centrifugation

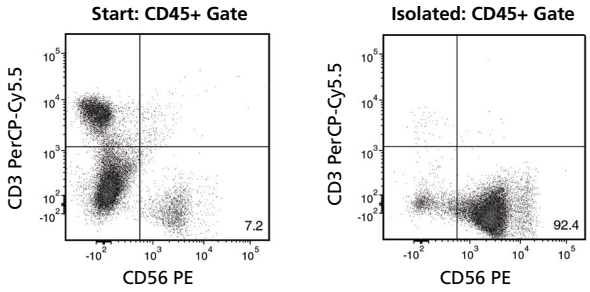


Figure 6. RosetteSep™ Human NK Cell Enrichment Cocktail (Catalog #15025)

Starting with whole peripheral blood, the NK cell content (gated on CD45+ cells) of the isolated fraction typically ranges from 80 - 98%. In the example above, the purities of the start and isolated fractions are 7.2% and 92.4%, respectively.

RosetteSep™ and SepMate™

Simplified and Standardized Cell Isolation

RosetteSep™ is easily combined with SepMate™ to rapidly and reproducibly isolate PBMC subsets from whole blood. By using the unique SepMate™ tube, sample throughput is increased and errors associated with improper sample layering are eliminated. This allows even users with minimal training to consistently perform cell isolation by density gradient centrifugation in a busy laboratory environment.

Human CD34+ Hematopoietic Progenitor Cell Isolation by Positive Selection

Applications

- Isolate human CD34+ cells from a variety of tissues.
- Obtain highly purified CD34+ cell populations for use in downstream assays.

Product Listing

Source	Product Name	Purity[1]	Capacity	Catalog #
Mobilized PBMCs, CBMCs, BMMCs	EasySep™ Human CD34 Positive Selection Kit II	93.5 ± 1.1%	For labeling up to 5 x 10^9 cells	17856[2], 17856RF
Mobilized Leukopak	EasySep™ Human CD34 Positive Selection Kit II	94.6 ± 2.4%	For labeling up to 1 x 10^{10} cells	100-1569
Whole Blood, Buffy Coat	EasySep™ Human Whole Blood CD34 Positive Selection Kit	26 - 41%	For labeling 75 mL whole blood (37 mL buffy coat)	18086
	EasySep™ Human Whole Blood CD34 Positive Selection Kit II			17879, 17879RF
	RoboSep™ Human Whole Blood CD34 Positive Selection Kit			18086RF
Whole Blood	Complete Kit for Human Whole Blood CD34+ Cells	79 - 95%	For labeling 120 mL whole blood	15086
	RoboSep™ Complete Kit for Human Whole Blood CD34+ Cells			15086RF
Fresh Cord Blood	EasySep™ Human Cord Blood CD34 Positive Selection Kit II	91 ± 9%	For labeling 1000 mL cord blood	17896[3,4]
	RoboSep™ Human Cord Blood CD34 Positive Selection Kit II			17896RF[3,4]
	EasySep™ Human Cord Blood CD34 Positive Selection Kit III	87 ± 12%		17897[4]
	RoboSep™ Human Cord Blood CD34 Positive Selection Kit III			17897RF[4]

PBMC: peripheral blood mononuclear cell; CBMC: cord blood mononuclear cell; BMMC: bone marrow mononuclear cell; hESC: human embryonic stem cell; hiPSC: human induced pluripotent stem cell.

For staining after positive selection with all Human CD34 Positive Selection kits, please use Anti-Human CD34, Clone 581 (Catalog #60013).

1. *Purities shown as either a range or mean ± SD. Purity data for 18056, 18086, and 15086 are reported as a percentage of viable CD45+ cells.*
2. *These kits are for use with fresh or previously frozen PBMC, BMMC, and previously frozen cord blood mononuclear cells. For isolation of CD34+cells from fresh cord blood, please use 18096RF, 17896, 17896RF, 17897, or 17897RF.*
3. *These kits are new versions of 18096 and 18096RF with improved performance.*
4. *For more information about choosing a CD34+ cell isolation kit for use with cord blood samples, please see our Technical Bulletin (Document #27003).*

For a full listing of cell isolation tools and reagents, visit ***www.stemcell.com/HSPCworkflow*** *under the "Cell Sourcing & Isolation" tab.*

Human Hematopoietic Progenitor Cell Isolation by Negative Selection

Applications

- Enrich human hematopoietic progenitors (i.e. CD34+ cells) by using monoclonal antibodies targeted to specific cell surface antigens to deplete unwanted mature cell types (i.e. lineage depletion).
- Obtain unlabeled progenitor-enriched cell populations, i.e. lineage negative (Lin-) cells.

Product Listing

Source	Product Name	Purity[1]	Capacity	Catalog #
Mobilized PBMCs, BMMCs	EasySep™ Human Progenitor Cell Enrichment Kit	42 ± 5 fold CD34+ cell enrichment (bone marrow)	For labeling up to 1 x 10^9 cells	19056
	RoboSep™ Human Progenitor Cell Enrichment Kit			19056RF
PBMCs, CBMCs	EasySep™ Human Progenitor Enrichment Kit with Platelet Depletion	50 - 75%	For labeling up to 1 x 10^9 cells	19356[2]
	RoboSep™ Human Progenitor Enrichment Kit with Platelet Depletion			19356RF[2]
Bone Marrow	RosetteSep™ Human Bone Marrow Progenitor Cell Pre-Enrichment Cocktail	25 ± 10 fold CD34+ cell enrichment	For labeling 40 mL bone marrow	15027
			For labeling 200 mL bone marrow	15067
Cord Blood	RosetteSep™ Human Hematopoietic Progenitor Cell Enrichment Cocktail II	77.5 ± 16.0%	1 x 10^9 cells	17936, 17936RF
	RosetteSep™ Human Hematopoietic Progenitor Cell Enrichment Cocktail	29 ± 9%	For labeling 40 mL cord blood	15026
			For labeling 200 mL cord blood	15066
	Complete RosetteSep™ Human Cord Blood Progenitor Enrichment Kit	29 ± 9%	For processing 500 mL cord blood	15276
Cord Blood	RosetteSep™ Human Cord Blood Debulking Cocktail	5 ± 1% (CD34+ cells)	For labeling 40 mL cord blood	15126[3]
			For labeling 200 mL cord blood	15166[3]

PBMC: peripheral blood mononuclear cell; BMMC: bone marrow mononuclear cell; CBMC: cord blood mononuclear cell.
For compatible staining antibodies, use 60013 Anti-Human CD34, 60018 Anti-Human CD45, 60026 Anti-Dextran.
1. Purities of CD34+ cells shown as either a range or mean ± SD. Purity of CD34+ cells for 19056 is reported relative to viable CD45+ cells in the start sample.
2. This product is designed for use with samples that contain large numbers of platelets.
3. This product is recommended for debulking cord blood of lineage positive cells prior to freezing.
For a full listing of cell isolation tools and reagents, visit ***www.stemcell.com/HSPCworkflow*** *under the "Cell Sourcing & Isolation" tab.*

Mouse Hematopoietic Progenitor Cell Isolation by Positive Selection

Applications

- Easily isolate mouse hematopoietic progenitor cells by positive selection.
- Select mouse hematopoietic progenitor cells using monoclonal antibodies to target specific cell surface antigens associated with progenitor cell phenotypes (e.g. SCA1+, cKIT+, AA4.1+).
- Achieve up to 97% purity using column-free immunomagnetic technology.

Cell Type	Source	Product Name	Purity	Capacity	Compatible Anti-Mouse Staining Antibodies	Catalog #
SCA1+ Cells	Bone Marrow	EasySep™ Mouse SCA1 Positive Selection Kit	87 - 97%	For labeling up to 2 x 10^9 cells	CD3e Antibody, Clone 145-2C11 (Catalog #60015); CD11b Antibody, Clone M1/70 (Catalog #60001); CD19 Antibody, Clone 6D5 (Catalog #60006); CD45R Antibody, Clone RA3-6B2 (Catalog #60019); Gr-1 Antibody, Clone RB6-8C5 (Catalog #60028); TER119 Antibody, Clone TER-119 (Catalog #60033)	18756
		RoboSep™ Mouse SCA1 Positive Selection Kit				18756RF
CD117+ (cKIT) Cells	Bone Marrow	EasySep™ Mouse CD117 (cKIT) Positive Selection Kit	88 - 95%	For labeling up to 2 x 10^9 cells	CD3e Antibody, Clone 145-2C11 (Catalog #60015); CD11b Antibody, Clone M1/70 (Catalog #60001); CD19 Antibody, Clone 6D5 (Catalog #60006); CD45R Antibody, Clone RA3-6B2 (Catalog #60019); Gr-1 Antibody, Clone RB6-8C5 (Catalog #60028); TER119 Antibody, Clone TER-119 (Catalog #60033)	18757
		RoboSep™ Mouse CD117 (cKIT) Positive Selection Kit				18757RF

Mouse Hematopoietic Progenitor Cell Isolation by Negative Selection

Applications

- Easily isolate untouched mouse hematopoietic progenitors (including Lin-SCA1+cKIT+ or LSK cells) by depleting unwanted mature cell types using monoclonal antibodies targeted to specific cell surface antigens expressed on these cells (i.e. lineage depletion).
- Efficiently obtain functional, unlabeled, progenitor-enriched cell populations, i.e. lineage negative (Lin-) cells.
- Achieve up to 84% purity using column-free immunomagnetic technology.

Cell Type	Source	Product Name	Purity	Capacity	Compatible Anti-Mouse Staining Antibodies	Catalog #
Hematopoietic Progenitor Cells	Bone Marrow	EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Kit	60 - 84%	For labeling up to 1 x 10^9 cells	CD117 Antibody, Clone 2B8 (Catalog #60025); Sca1 Antibody, Clone E13-161.7 (Catalog #60032); CD3e Antibody, Clone 145-2C11 (Catalog #60015); CD11b Antibody, Clone M1/70 (Catalog #60001); CD19 Antibody, Clone 6D5 (Catalog #60006); CD45R Antibody, Clone RA3-6B2 (Catalog #60019); Gr-1 Antibody, Clone RB6-8C5 (Catalog #60028) TER119 Antibody, Clone TER-119 (Catalog #60033)	19856
		RoboSep™ Mouse Hematopoietic Progenitor Cell Isolation Kit				19856RF

For a full listing of cell isolation tools and reagents for mouse cells, visit ***www.stemcell.com/hspc-mouse-workflow****.*

EasySep™ RBC Depletion Agent

Gentle, Lysis-Free Leukocyte Purification

In many laboratories, the standard protocols for obtaining leukocytes from human whole blood samples involve density gradient centrifugation or lysing red blood cells (RBCs) with ammonium chloride. However, these methods can be time consuming, be difficult to automate, and leave residual cell debris that may alter cellular function or interfere with downstream assays.

EasySep™ RBC Depletion Reagent immunomagnetically depletes RBCs without lysis, washes, or centrifugation steps. The resulting highly purified leukocytes are untouched and ready for downstream applications, including cell culture, RNA isolation, or enzyme activity testing. EasySep™ RBC Depletion Reagent can also be used on cord blood, bone marrow, buffy coats, and leukapheresis products to meet all of your laboratory's needs for isolating leukocytes.

Why Use EasySep™ RBC Depletion Reagent?

GENTLE. Avoid lysis buffer, centrifugation, or additional washing steps.

RELIABLE. Deplete 99.9% of RBCs immunomagnetically without leaving additional debris that may interfere with downstream assays.

FAST. Obtain leukocytes in as little as 9 minutes.

CONVENIENT. Automate blood sample processing with RoboSep™ instruments to increase laboratory throughput.

EasySep™ RBC Depletion Reagent

Product Name	Volume Processed	Catalog #
EasySep™ RBC Depletion Reagent	100 mL	18170
EasySep™ RBC Depletion Reagent for RoboSep™	100 mL	18170RF

To learn more, visit **www.stemcell.com/RBCdepletion**.

HetaSep™

Erythrocyte Aggregation

HetaSep™

Product Name	Catalog #	Size
HetaSep™	07806	20 mL
	07906	100 mL

The presence of large numbers of RBCs in a colony-forming unit (CFU) assay prevents hematopoietic colonies from being accurately visualized either manually or using STEMvision™ (Figure 7). RBCs must be removed from fresh cord blood, bone marrow, and mobilized peripheral blood samples (whether whole or processed), before performing the CFU assay.

HetaSep™ is an erythrocyte aggregation agent used to quickly separate nucleated cells from RBCs. It is based on the principle that aggregated erythrocytes settle much faster than dispersed cells.

The HetaSep™ procedure does not affect the number of progenitor cells; 97% of CFUs are recovered in the RBC-cleared sample (Figure 8). HetaSep™-mediated RBC depletion requires only 50 µL of sample and is quick, making it easy to incorporate into an institution's workflow.

For more information, see the HetaSep™ Protocol Technical Bulletin (Document #29541) or visit **www.stemcell.com/hetasep_protocol.**

Why Use HetaSep™?

ACCURATE. Increases the accuracy of colony counting by removing RBC background.

CONSISTENT. Recover > 97% of colonies.

FAST. Easy to perform, no centrifuge needed. Can be performed with only 50 µL of sample.

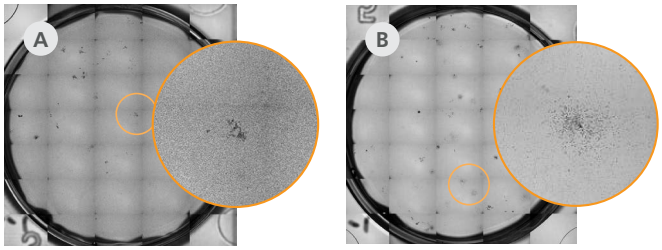


Figure 7. STEMvision™ Images of 7-Day CFU Assays of Fresh Cord Blood Samples Plated in MethoCult™ Express

(A) Cord blood samples without RBC removal using HetaSep™. There is unacceptable background for a CFU assay. Note that fewer colonies are visible due to increased RBCs in culture. (B) Cord blood sample with prior removal of RBCs using HetaSep™. Acceptable background (minimal RBCs) for a CFU assay.

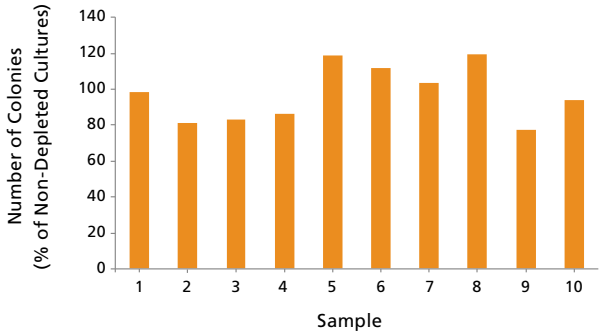


Figure 8. An Average of 97% of Colony-Forming Cells Are Recovered Following RBC Depletion with HetaSep™

Cord blood samples (n = 10) were split into two volumes, one of which was plated in a CFU assay without RBC depletion, and the other was plated after undergoing RBC depletion using the HetaSep™ protocol. Each sample type was plated in duplicate. CFU assays were counted manually and the percent recovery of colonies in each RBC-depleted fraction was calculated relative to results of CFU assays of non-depleted cells from the same donor.

Cryopreservation Media

cGMP Freezing and Preservation Media Formulated with USP-Grade Components

The cryopreservation and subsequent storage of hematopoietic cells is an important step in hematopoietic stem and progenitor cell research. STEMCELL Technologies' suite of cGMP, protein-free, and serum-free cryopreservation products are designed to maintain high viability and maximize cell recovery after long-term storage. For short-term storage and shipping of cells, HypoThermosol® products preserve cells at 2 - 8°C.

CryoStor® Freezing Media

- **Cell Types:** blood cells derived from peripheral blood.
- Designed to mitigate temperature-induced molecular stress responses during freezing and thawing.
- CryoStor® is pre-formulated with 2%, 5%, or 10% USP-grade DMSO.
- U.S. FDA Drug Master File

HypoThermosol® FRS Preservation Media

- **Cell Types:** all cells and tissues, including hematopoietic stem and progenitor cells.
- HypoThermosol® FRS is designed for short-term storage and/or shipment of cells at 2 - 8°C rather than at cryogenic temperatures.
- U.S. FDA Drug Master File

Video

Freeze Human PBMCs with CryoStor® CS10

www.stemcell.com/Cryopreserve-Primary-Cells-With-Cryostor

BloodStor® Freezing Media

- **Cell Types:** blood cells derived from peripheral blood, and bone marrow.
- BloodStor® 55-5 is preformulated with 55% (w/v) DMSO USP, 5% (w/v) Dextran-40 USP, and water for injection- (WFI) quality water.
- BloodStor® 100 contains 100% (w/v) DMSO USP.

Product Listing

Product Name	Catalog #	Unit Size
CryoStor® CS10	100-1061	100 mL
	07955	100 mL bag
	07940	1000 mL bag
	07931	5 x 16 mL vials
	07959	5 x 10 mL vials
	07952	16 x 10 mL vials
CryoStor® CS5	07933	100 mL
	07953	100 mL bag
	07949	5 x 10 mL vials
CryoStor® CS2	07932	100 mL
HypoThermosol® FRS	07935	100 mL
	07936	500 mL
	07945	500 mL bag
	07934	16 x 10 mL vials
BloodStor® 55-5	07937	16 x 7.2 mL vials
BloodStor® 100	07951	50 mL
	07939	100 mL
	07938	5 x 100 mL

StemSpan™ Expansion Media & Supplements

Expansion and Differentiation of Hematopoietic Stem and Progenitor Cells

Cell Sourcing & Isolation | **Expansion & Differentiation** | Analysis

StemSpan™ Expansion Media include serum-free, xeno-free, and animal component-free formulations, as well as StemSpan™-AOF, the first commercially available animal origin-free medium for culturing hematopoietic stem and progenitor cells (HSPCs), manufactured under relevant cGMPs. StemSpan™ media do not contain any cytokines, allowing the flexibility to prepare a medium to meet the specific requirements of an experiment. StemSpan™ Expansion Supplements are pre-mixed cocktails of recombinant human cytokines and other additives formulated to selectively expand CD34+ HSPCs and/or stimulate their differentiation into mature cells of specific lineages when added to a StemSpan™ medium.

Applications

- Ex vivo expansion of HSPCs.[1,2]
- Identification of novel regulators of HSPCs.[3-7]
- Production of large numbers of mature blood cells in vitro.[3,8-9]
- Generation of target cells for reprogramming to make induced pluripotent stem cells.[10]
- Gene transfer into HSPCs.[11,12]

Serum-Free Expansion Media

Medium	Product Name	Catalog # (Size)	Recommended For	Components
Serum-Free	StemSpan™ SFEM	09600 (100 mL) 09650 (500 mL)	Culture of human HSPCs Culture of mouse, rat, and non-human primate HSPCs	Pre-tested BSA, insulin, transferrin, and supplements in IMDM
	StemSpan™ SFEM II	09605 (100 mL) 09655 (500 mL)	Culture and expansion of human HSPCs Production of mature blood cells by expansion and lineage-specific differentiation of human HSPCs	
Animal Origin-Free	StemSpan™-AOF	100-0130 (500 mL)	Culture and expansion of human HSPCs in the absence of human- or animal-derived components when higher compliance is required, e.g. for cell therapy manufacturing	Recombinant and synthetic components in IMDM
Xeno-Free	StemSpan™-XF	100-0073 (500 mL)	Culture and expansion of human HSPCs in the absence of animal-derived components*	Human-derived or recombinant human proteins in IMDM
Animal Component-Free	StemSpan™-ACF Erythroid Expansion Medium	09860 (500 mL)	Culture and expansion of human erythroid cells in the absence of human- or animal-derived components	Recombinant proteins and synthetic components only

BSA: bovine serum albumin; IMDM: Iscove's Modified Dulbecco's Medium.
*Contains pre-tested human-derived and recombinant human proteins

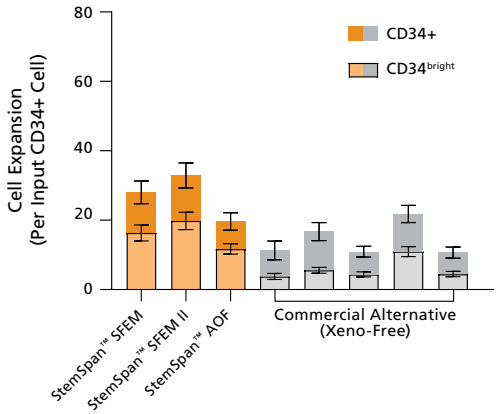


Figure 9. StemSpan™ Media Support Greater Expansion of Human CD34+ and CD34^{bright} Cells Than Other Commercial Media

Purified CB-derived CD34+ cells were cultured for 7 days in select StemSpan™ media (StemSpan™ SFEM, StemSpan™ SFEM II, StemSpan™-XF, or StemSpan™-AOF, orange bars), and in five xeno-free media formulations from other suppliers (Commercial Alternative, gray bars) including (in random order) CTS™ StemPro™ HSC (Thermo), SCGM (Cellgenix), X-VIVO™ 15 (Lonza), Stemline™ II (Sigma), and StemPro™-34 (Thermo). All media were supplemented with StemSpan™ CD34+ Expansion Supplement and UM171*. Cell expansion of viable CD34+ and CD34^{bright} cells in culture were measured based on viable cell counts and flow cytometry results. StemSpan™ showed significantly higher expansion of CD34+ and CD34^{bright} cells ($P < 0.05$ when comparing StemSpan™ SFEM II to five media from other suppliers, calculated using a one-way ANOVA followed by Dunnett's post hoc test) and StemSpan™-AOF, the only animal origin-free formulation, showed equivalent performance to all xeno-free commercial alternatives tested. Data shown are mean ± SEM (n = 8).

Note: Data for StemSpan™-AOF shown were generated with the original phenol red-containing version, StemSpan™-ACF (Catalog #09855). However, internal testing showed that the performance of the new phenol red-free, cGMP-manufactured version, StemSpan™-AOF (Catalog #100-0130), was comparable.

**Similar results are expected when using UM729 (Catalog #72332) prepared to a final concentration of 1µM. For more information, including data comparing UM171 and UM729, see Fares et al., 2014.*

StemSpan™-AOF

GMP-Compliant Medium for Cell Therapy Development

Hematopoietic stem and progenitor cells (HSPCs) are widely used in cell and gene therapy applications. When culturing HSPCs for cell therapy development, it is important to minimize risk and variability in your cell culture medium to ensure consistent, reproducible performance and safety. Whether you are performing fundamental research or ready to transition to the clinic, StemSpan™-AOF medium helps minimize the risk of viral contamination in your cell therapy development. Choosing animal origin-free (AOF) cell culture conditions can facilitate a smoother pathway to the clinic by helping you avoid regulatory roadblocks. StemSpan™-AOF is also manufactured under relevant cGMPs, ensuring the highest quality and consistency for reproducible results.

StemSpan™-AOF for Gene Editing Applications

The ability to genetically manipulate HSPCs has significantly advanced our understanding of the mechanisms that regulate hematopoiesis and is contributing to the development of novel cellular therapies. Using a medium that supports genome editing of hematopoietic cells can help take your cell therapy development to the next level. See how StemSpan™-AOF supports optimal culture conditions for HSPC maintenance and expansion in CRISPR-Cas9 gene editing applications:

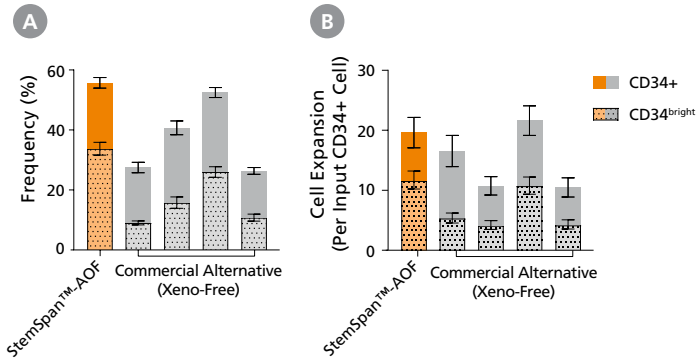


Figure 10. StemSpan™-AOF Supports Equivalent or Greater Expansion of Human CD34+ and CD34^{bright} Cells Compared to Other Commercial Media

Purified cord blood-derived CD34+ cells were cultured for 7 days in StemSpan™-AOF (orange bar) and in four alternative commercial media (gray bars). Each medium was supplemented with StemSpan™ CD34+ Expansion Supplement and 175 nM UM171*. The (A) frequency and (B) cell expansion of viable CD34+ and CD34^{bright} cells in culture were measured based on viable cell counts and flow cytometry results. StemSpan™-AOF, the only animal origin-free formulation, showed equivalent performance to all xeno-free alternative media tested.

Why Use StemSpan™-AOF for Cell Therapy Development?

SAFE. Minimize the risk of viral contamination by using a medium that does not contain any primary or secondary raw materials derived from animals.

ROBUST. Ensure consistency in your experiments by using serum-free and animal origin-free culture conditions.

FLEXIBLE. Customize your cell culture conditions by adding StemSpan™ Expansion Supplements, individual cytokines, or additives to suit your specific cell therapy development needs.

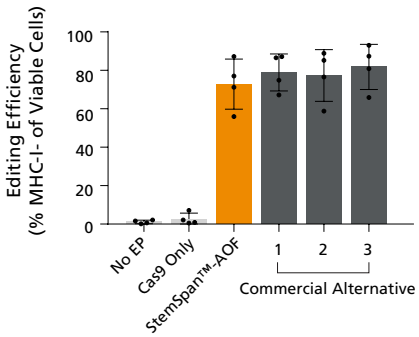


Figure 11. Human CD34+ Cells Cultured in StemSpan™-AOF Show Equivalent Gene Editing Efficiency Compared with Alternative Media

Cells cultured for 2 days in either StemSpan™-AOF (orange bar) or xeno-free alternative commercial media (gray bars), each supplemented with StemSpan™ CD34+ Expansion Supplement and 175 nM UM171*, were electroporated with CRISPR-Cas9 RNP complexes containing crRNA:tracrRNA targeting β2 Microglobulin (B2M). Non-electroporated (No EP) cells and cells electroporated with Cas9 without gRNA (Cas9 Only) were cultured in StemSpan™ SFEM II supplemented with StemSpan™ CD34+ Expansion Supplement plus 175 nM UM171*. B2M knockout efficiency (% MHC-I- viable cells) was monitored by flow cytometry using a fluorophore-conjugated anti-MHC-I antibody.

**Similar results are expected when using UM729 (Catalog #72332) prepared to a final concentration of 1 µM. For more information, including data comparing UM171 and UM729, see Fares et al. Science, 2014.*

In Vivo Engraftment of HSPCs

One of the best assays to determine the quality of a hematopoietic cell therapy product is evaluation of its engraftment and multilineage differentiation potential after intravenous injection into immunodeficient mice (e.g. NOD scid gamma (NSG) mice). The "stemness" of HSPCs can be affected by many parameters, such as cell processing methods and culture conditions used for expansion and gene editing, which may impact the ability of the cells to successfully engraft. StemSpan™-AOF, the only animal origin-free cGMP medium on the market, supports multilineage engraftment of CD34+ cells at equivalent or higher levels when compared to uncultured cells.

See how StemSpan™-AOF supports the engraftment and expansion of cord blood-derived CD34+ cells in NSG mouse recipients:

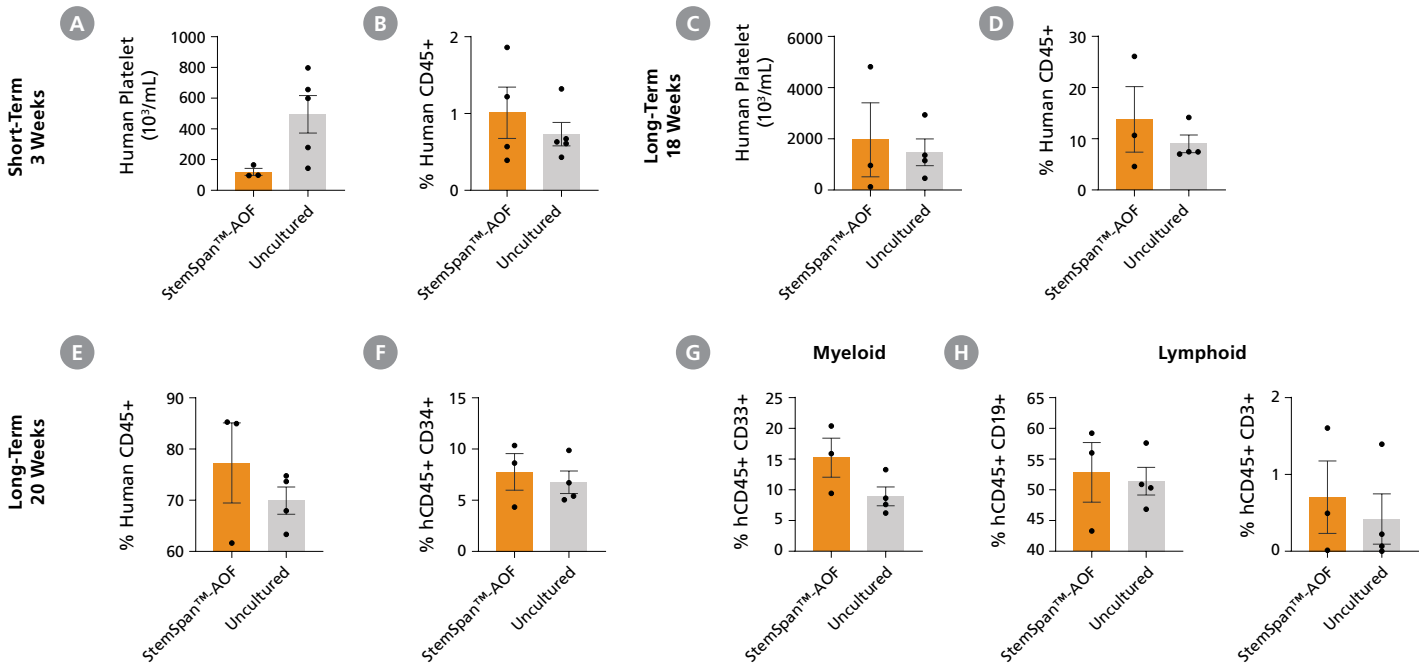


Figure 12. StemSpan™-AOF-Expanded Cord Blood CD34+ Cells Engraft in NSG Mouse Recipients

Purified cord blood-derived CD34+ cells were cultured for 7 days in StemSpan™-AOF supplemented with StemSpan™ CD34+ Expansion Supplement and UM729 (1 µM). After 7 days of expansion, progeny of 10,000 fresh or uncultured CD34+ cells were transplanted in sub-lethally irradiated NSG mice. (A-D) The number of human platelets and the frequency of human cells expressing the pan-leukocyte marker CD45 were measured in peripheral blood at 3 and 18 weeks post-transplantation. Data shown are mean ± SEM (n = 3 - 5 mice). (A) At 3 weeks, engraftment of human platelets was lower in recipients of cells cultured in StemSpan™-AOF than in recipients of uncultured cells. (C) At week 18, there were no significant differences in platelet engraftment between the expanded and uncultured cells. (B,D) Human CD45+ cell frequencies in recipients of cells expanded in StemSpan™-AOF were similar to those in recipients of uncultured cells. (E-H) At week 20, long-term multilineage engraftment was measured in bone marrow of transplanted NSG mice. Data shown are mean ± SEM (n = 3 - 4 mice). (E,F) Recipients of StemSpan™-AOF expanded cells showed similar frequencies of human CD45+ and CD34+ cells in the mouse bone marrow compared to recipients of uncultured cells. (G,H) Cells expanded with StemSpan™-AOF showed similar levels of myeloid (CD45+CD33+) and lymphoid (CD45+19+ B cells and CD45+CD3+ T cells) engraftment relative to uncultured cells.

Taking Your Research to the Clinic?

STEMCELL's Services for Cell Therapy program has a team of experts who can help support your regulatory filing by providing custom solutions such as quality documentation, additional product testing, and customized product manufacturing. To learn more about how we can support your preclinical and clinical research needs, visit us at **www.stemcell.com/services/cell-therapy.html**.

Resource

Visit the Cell Therapy Learning Center
www.stemcell.com/hsc-learning

CD34 Expansion Supplements

Investigators studying hematopoiesis require standardized culture media and cytokines to promote the proliferation and/or lineage-specific differentiation of HSPCs from human bone marrow (BM), cord blood (CB), and other tissues. StemSpan™ media require the addition of cytokines to promote HSPC proliferation and differentiation. The choice of cytokines and supplements depends on the objective of the experiment, i.e. on the desired numbers of specific cell types to be generated in vitro.

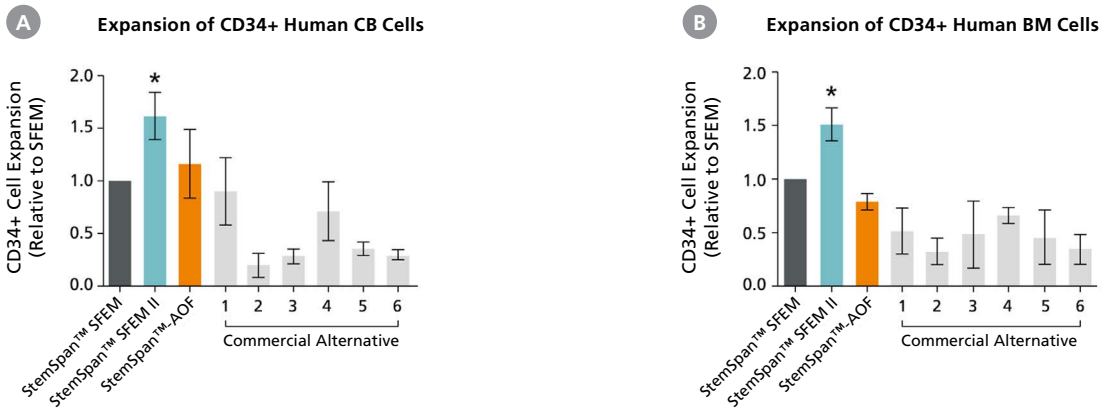


Figure 13. StemSpan™ SFEM II Serum-Free Expansion Medium Containing CC100 Cytokine Cocktail Supports Greater Expansion of Human CD34+ Cells than Other Media Tested

Expansion of CD34+ cells, normalized relative to the values obtained in StemSpan™ SFEM medium (dark gray bars), after culturing purified CD34+ CB (A, n = 6) or bone marrow (BM) (B, n = 3) cells for 7 days in StemSpan™ SFEM, SFEM II (blue bars) and AOF (orange bars), and six media from other commercial suppliers (light gray bars). All media were supplemented with StemSpan™ CC100 Cytokine Cocktail (Catalog #02690). Vertical lines indicate 95% confidence limits, i.e. the range within which 95% of results fall. The numbers of CB and BM cells produced in StemSpan™ SFEM II were significantly higher than in all other media, with the exception of CB cells cultured in StemSpan™-AOF (*$p<0.05$, paired t-test).

Note: Data for StemSpan™-AOF shown were generated with the original phenol red-containing version, StemSpan™-ACF (Catalog #09855). However, internal testing showed that the performance of the new phenol red-free, cGMP version, StemSpan™-AOF (Catalog #100-0130), was comparable.

CD34+ Cell Expansion Supplements

Product Name	Catalog # (Size)	Product Features	Components
StemSpan™ CC100	02690 (1 mL, 100X)	Contains both early- and late-acting cytokines Stimulates the production of large numbers of human hematopoietic cells, including CD34+ progenitor cells	rhFlt3L, rhSCF, rhIL-3, rhIL-6
StemSpan™ CC110	02697 (1 mL, 100X)	Contains early-acting cytokines Stimulates similar expansion of CD34+ cells as CC100, but with higher purity	rhFlt3L, rhSCF, rhTPO
StemSpan™ CD34+ Expansion Supplement	02691(10 mL, 10X)	Recommended for selective expansion of human CD34+ HSPCs Stimulates greater CD34+ cell expansion compared to CC100 and CC110	rhFlt3L, rhSCF, rhIL-6, rhTPO Other additives

rh: recombinant human. For a full listing of products for expansion and differentiation of HSPCs, visit ***www.stemcell.com/HSPCworkflow****.*

Expansion and Lineage-Specific Differentiation Supplements and Kits

Cell Type	Product Name	Catalog # (Size)	Product Features	Components
Erythroid Progenitor Cells	StemSpan™ Erythroid Expansion Supplement	02692 (1 mL, 100X)	Stimulates the production of human erythroid cells by expansion and lineage-specific differentiation of human HPCs	rhSCF, rhIL-3, rhEPO
Megakaryocytes	StemSpan™ Megakaryocyte Expansion Supplement	02696 (1 mL, 100X)	Stimulates the production of human megakaryocytes by expansion and lineage-specific differentiation of human HPCs	rhSCF, rhTPO, rhIL-6, rhIL-9
Granulocytes	StemSpan™ Myeloid Expansion Supplement	02693 (1 mL, 100X)	Stimulates the production of human granulocytes by expansion and lineage-specific differentiation of human HPCs	rhSCF, rhTPO, rhG-CSF, rhGM-CSF
Monocytes	StemSpan™ Myeloid Expansion Supplement II	02694 (1 mL, 100X)	Stimulates the production of human monocytes by expansion and lineage-specific differentiation of human HPCs	rhFlt3L, rhSCF, rhTPO, rhM-CSF, rhGM-CSF and supplements
T Cells	StemSpan™ T Cell Generation Kit	09940 (Kit, various)	Stimulates the production of human T cells by expansion and lineage-specific differentiation of human HSPCs in stroma-free conditions	• SFEM II • Lymphoid Progenitor Expansion Supplement (10X) • Lymphoid Differentiation Coating Material (100X) • T Cell Progenitor Maturation Supplement (10X)
NK Cells	StemSpan™ NK Cell Generation Kit	09960	Stimulates the production of human NK cells by expansion and differentiation of human CD34+ HSPCs in stroma-free conditions	• SFEM II • Lymphoid Progenitor Expansion Supplement (10X) • Lymphoid Differentiation Coating Material (100X) • NK Cell Differentiation Supplement (100X)
B Cells	StemSpan™ B Cell Generation Kit	100-1250	Stimulates the production of B cells and antibody-secreting cells (ASCs) of human CD34+ HSPCs in serum- and feeder-free conditions	• StemSpan™ SFEM II • StemSpan™ B Cell Differentiation Supplement 1 (20X) • StemSpan™ B Cell Differentiation Supplement 2 (20X) • StemSpan™ B Cell Differentiation Supplement 3 (20X) • StemSpan™ B Cell Differentiation Supplement 4 (20X)
Myeloid Leukemic Cells	StemSpan™ Leukemic Cell Culture Kit	09720 (Kit, various)	Stimulates the production of human myeloid leukemia cells, allowing users to expand, culture, and use malignant cells for drug screening	• SFEM II • CD34+ Expansion Supplement • UM729

rh: recombinant human. For a full listing of products for expansion and differentiation of HSPCs, visit ***www.stemcell.com/HSPCworkflow****.*

Differentiation of Human Progenitor Cells in Culture

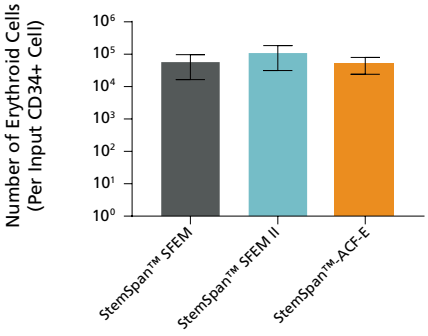


Figure 14. Production of Erythroid Cells from Human CB-Derived CD34+ Cells Cultured in StemSpan™ Media Containing StemSpan™ Erythroid Expansion Supplement

Average numbers of erythroid cells generated after culturing purified CD34+ CB cells (n = 5) for 14 days in StemSpan™ SFEM gray bar), SFEM II (blue bar), or StemSpan™-ACF Erythroid Expansion Medium (ACF-E, orange bar) media containing StemSpan™ Erythroid Expansion Supplement (Catalog #02692). Shown are the number of erythroid cells that express CD71 and/or Glycophorin A (GlyA) per input CD34+ cell.

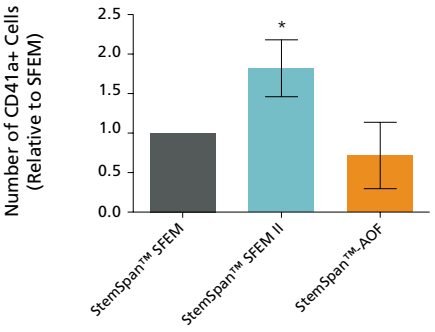


Figure 15. Comparison of Megakaryocyte Expansion in Different StemSpan™ Media Containing StemSpan™ Megakaryocyte Expansion Supplement

Average numbers of CD41+ megakaryocytic cells normalized relative to the values obtained in StemSpan™ SFEM (gray bar) after culturing purified CD34+ cord blood cells (n=6) for 14 days in StemSpan™ SFEM, SFEM II (blue bar), and AOF (orange bar) media containing StemSpan™ Megakaryocyte Expansion Supplement. Vertical lines indicate 95% confidence limits, the range within which 95% of results typically fall.

**The numbers of CD41a+ cells were significantly higher in SFEM II ($p<0.01$, paired t-test, n=6) compared to SFEM and AOF medium.*

Note: Data for StemSpan™-AOF shown were generated with the original phenol red-containing version StemSpan™-ACF (Catalog #09855). However internal testing showed that the performance of the new phenol red-free, cGMP-manufactured version, StemSpan™-AOF (Catalog #100-0130) was comparable.

Lymphoid Expansion and Differentiation

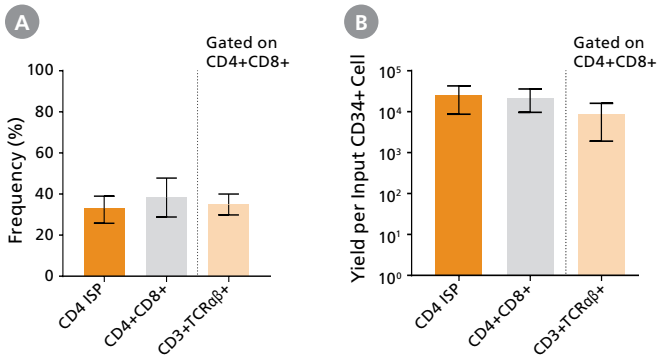


Figure 16. Frequency and Yield of CD4 Immature Single-Positive and CD4+CD8+ Double-Positive Cells After 42 Days of Culture

CB-derived CD34+ cells were cultured with the StemSpan™ T Cell Generation Kit (Catalog #09940) for 42 days. The average (A) frequency and (B) yield of CD4 immature single-positive (ISP), CD4+CD8+ double-positive, and CD3+TCRαβ+-expressing double-positive cells (CD4+CD8+CD3+TCRαβ+) are shown. Vertical lines indicate 95% confidence interval (n = 31). On average, 38% of the total viable population were CD4+CD8+ double-positive, of which 35% co-expressed CD3 and TCRαβ. The yield of total double-positive cells per input CD34+ cell was ~23,000.

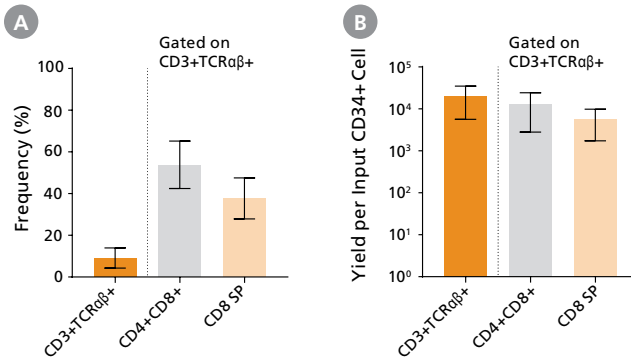


Figure 17. Frequency and Yield of CD8 Single-Positive T Cells After 49 Days of Culture

CD4+CD8+ double-positive cells were further matured into CD8 single-positive T cells by culturing for an additional 7 days in StemSpan™ SFEM II with T Cell Progenitor Maturation Supplement (Catalog #09930), IL-15 (Catalog #78031), and ImmunoCult™ CD3/CD28/CD2 T Cell Activator (Catalog #10970). The average (A) frequency and (B) yield of CD3+TCRαβ+-expressing cells and their subsets are shown. Vertical lines indicate 95% confidence interval (n = 12). On average, 54% of the CD3+TCRαβ+ cells were CD4+CD8+ double-positive and 38% were CD8 single-positive (CD4-CD8+).

Small Molecules for Human Hematopoietic Stem and Progenitor Cell Research

UM729 and StemRegenin 1

UM729 and StemRegenin 1 (SR1) are two small molecules that have been found to enhance the self-renewal and expansion of human hematopoietic stem and progenitor cells in vitro.[13-15] UM729 is a pyrimido-[4,5-b]-indole derivative that acts differently than other small molecule stimulators of hematopoiesis, such as the aryl hydrocarbon receptor (AhR) antagonist SR1.[13-15] UM729 was originally discovered in a screen of compounds capable of promoting human CD34+ cell expansion and later underwent structure-activity relationship optimization to develop UM171.[14-15] Additionally, UM729 and SR1 have been shown to cooperate in culture, resulting in an additive effect in preventing the differentiation of primary human acute myeloid leukemia (AML) cells.[15]

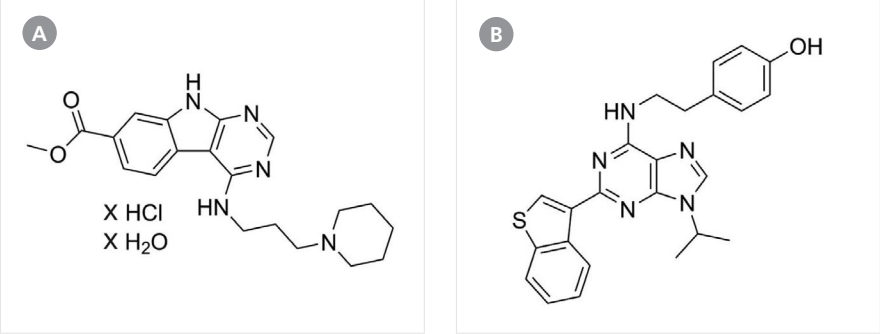


Figure 18. Chemical Structure of UM729 and StemRegenin 1

(A) UM729: Methyl 4-((3-(piperidin-1-yl)propyl)amino)-9H-pyrimido[4,5-b] indole-7-carboxylate
(B) StemRegenin 1 (SR1): 4-[2-[[2-benzo[b]thien-3-yl-9-(1-methylethyl)-9H-purin-6-yl]amino]ethyl]-phenol

Product Listing

Product Name	Catalog #	Size	Pathway / Target	Applications
UM729	72332	250 µg	Pyrimido-indole derivative that enhances HSC self-renewal in vitro	• Expansion of human HSCs in culture • Maintenance of LSCs in combination with SR1
StemRegenin 1	72342 72344	1 mg 5 mg	Aryl hydrocarbon receptor (AhR) antagonist	• Maintenance and expansion of human HSPCs in culture • Differentiation of human CD34+ HPCs into functional dendritic cells • Maintenance of LSC activity in culture when combined with UM729
StemRegenin 1 (Hydrochloride)	72352 72354	1 mg 5 mg		
StemSpan™ HSC Plus Supplement	100-1694	1 mL	Small molecule cocktail that enhances HSPC expansion in vitro	• Selective expansion of primitive HSPCs, including CD34+CD45RA-CD90+CD133+EPCR+ cells in liquid cultures initiated with CD34+ cells or purified CD34+ subsets isolated from cord blood, mobilized peripheral blood, or bone marrow

HPCs: hematopoietic progenitor cells; HSCs: hematopoietic stem cells; HSPCs: hematopoietic stem and progenitor cells; LSCs: leukemic stem cells.

StemSpan™ HSC Plus Expansion Supplement

StemSpan™ HSC Plus Supplement contains a combination of small molecules formulated to selectively promote the expansion of hematopoietic stem and progenitor cells (HSPCs) in cultures of human CD34+ cells or purified CD34+ subsets enriched for hematopoietic stem cells (HSCs), when added to the culture medium containing appropriate cytokines.

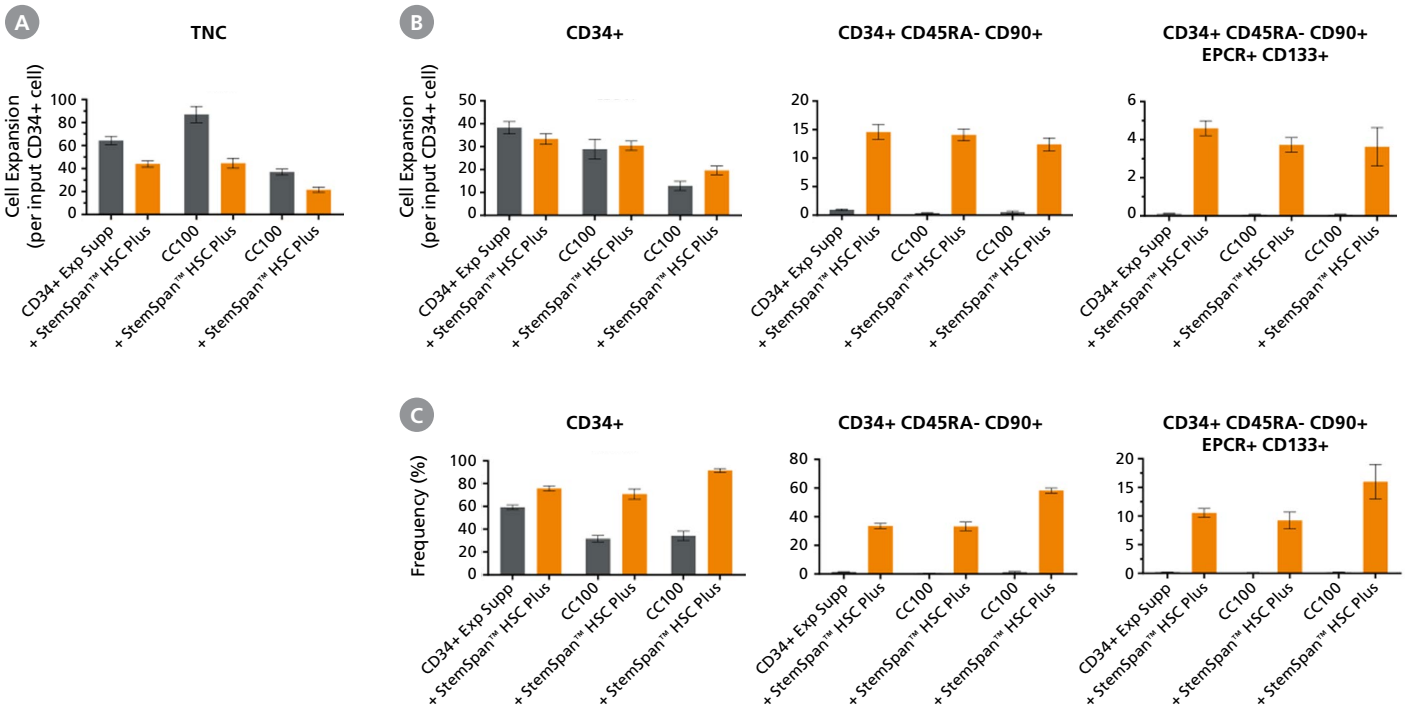


Figure 19. Expansion of Primitive HSPCs Is Enhanced by the Addition of StemSpan™ HSC Plus Supplement to Cytokine-Containing Medium

Purified CD34+ cells derived from cord blood (CB) were cultured at a concentration of 10,000 cells per mL in StemSpan™ SFEM II medium supplemented with one of the StemSpan™ cytokine-based supplements alone (CD34 Expansion Supplement, CC100, or CC110; gray bars), or together with StemSpan™ HSC Plus supplement (orange bars). After 7 days of culture, flow cytometry was used to analyze the (A) total nucleated cell (TNC) expansion, (B) expansion of CD34+ cell subsets, and (C) frequency of CD34+ HSPC subsets: CD34+, CD34+CD45RA-CD90+, and CD34+CD45RA-CD90+EPCR+CD133+. StemSpan™ HSC Plus Supplement promoted greater expansion of CD34+ CD45RA-CD90+ and CD34+CD45RA-CD90+CD133+EPCR+ HSPC subsets compared to cytokine-based supplements alone. Each subsequent cell subset was progressively more enriched for phenotypic stem/progenitor cells. Data shown are ± SEM (n = 24 for the CD34 Expansion Supplement dataset and n = 9 for both the CC100 and CC110 datasets).

STEMdiff™ Kits for Hematopoietic Differentiation of Pluripotent Stem Cells

Generate Hematopoietic Progenitor Cells, Immune Cells, and Blood Cells from Human Embryonic Stem and Induced Pluripotent Stem Cells

The STEMdiff™ Hematopoietic Kit consists of serum-free basal medium and supplements designed for the generation of hematopoietic progenitor cells (HPCs). Optimized for a standardized, 12-day differentiation protocol, this kit supports robust differentiation of human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs). HPCs generated in these cultures can be identified by the expression of CD34 and CD45, and by the ability to form hematopoietic colonies of multiple lineages in colony-forming unit (CFU) assays with MethoCult™ medium.

The resulting HPCs may be used for downstream assays or quantified in a CFU assay with MethoCult™ SF H4636 (Catalog # 04636) medium, designed specifically for use with hPSC-derived HPCs, or MethoCult™ H4435 (Catalog #04435) Enriched medium. HPCs generated with the STEMdiff™ Hematopoietic Kit may be further differentiated using the STEMdiff™ Microglia Differentiation Kit or STEMdiff™ Monocyte Kit. HPCs and downstream cells in the erythroid or megakaryocyte lineages may be obtained directly using the STEMdiff™ Erythroid or Megakaryocyte Kits, and HPC and immune cell types in the lymphoid lineages may be obtained using the STEMdiff™ NK and T Cell Kits.

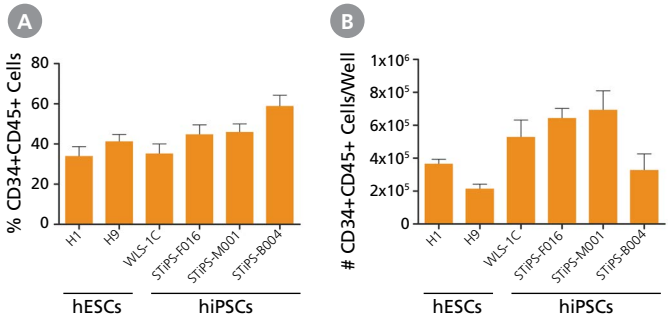


Figure 20. Efficient and Robust Generation of CD34+CD45+ HPCs

hESCs and hiPSCs were cultured for 12 days in single wells of 12-well plates using the STEMdiff™ Hematopoietic Kit. At the end of the culture period, cells in suspension were harvested, stained, and analyzed by flow cytometry for the expression of hematopoietic cell surface markers CD34 and CD45.
(A) Percentages and (B) total numbers of CD34+CD45+ cells in cultures of hESCs or hiPSCs are shown for 6 cell lines. Data shown as mean + SEM; n ≥ 3.

Why Use the STEMdiff™ Hematopoietic Kit?

CONSISTENT. Reduce variability with a serum- and feeder-free formulation.

EASY-TO-USE. Produce HPCs in suspension for easy harvest with a simple monolayer protocol.

RAPID. Generate HPCs in 12 days.

HIGH YIELD. Generate 4 - 18 million CD34+CD45+ HPCs with one kit.

FLEXIBLE. Generate HPCs across multiple hESC and hiPSC lines.

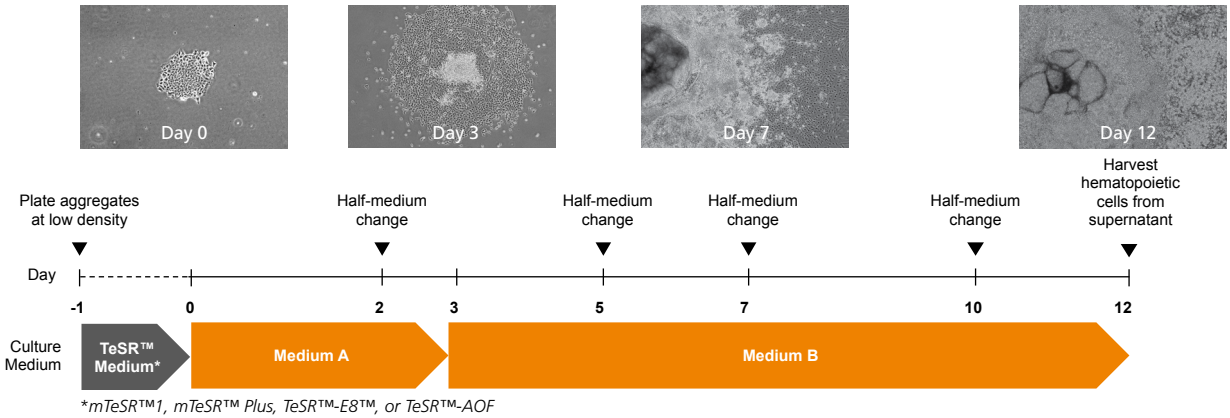


Figure 21. Schematic for Hematopoietic Differentiation

One day before the differentiation protocol, hPSC colonies are harvested and seeded as small aggregates (100 - 200 µm in diameter) at 10 - 20 aggregates/cm^2 in a TeSR™ maintenance medium (mTeSR™ Plus, mTeSR™1, or TeSR™-E8™). After one day, TeSR™ medium is replaced with Medium A to begin inducing the cells toward a mesoderm-like state (Day 0). On Day 2, a half-medium change is performed with fresh Medium A. On Day 3, the medium is changed to Medium B with half-medium changes on Days 5, 7, and 10 to promote further hematopoietic differentiation. Typically, by Day 12, large numbers of HPCs can be harvested from the culture supernatant.

Learn more at **www.stemcell.com/STEMdiffHeme**

STEMdiff™ Megakaryocyte Kit

STEMdiff™ Megakaryocyte Kit is designed for the serum-free and feeder-free differentiation of hESCs and hiPSCs to megakaryocytes expressing CD41a and CD42b. This optimized two-dimensional and two-stage protocol is capable of generating high yields of megakaryocytes per hESC or hiPSC in 17 days. The resulting megakaryocytes show high ploidy and platelet-shedding ability and are also amenable to large-scale culture.

Why Use STEMdiff™ Megakaryocyte Kit?

CONSISTENT. Reduce variability with a serum- and feeder-free formulation.

HIGH YIELD. Expand hESCs and hiPSCs by > 400-fold during megakaryocyte differentiations, with > 70% yield for CD41a and CD42b megakaryocytes.

EASY-TO-USE. Optimized two-dimensional protocol produces megakaryocytes in 17 days.

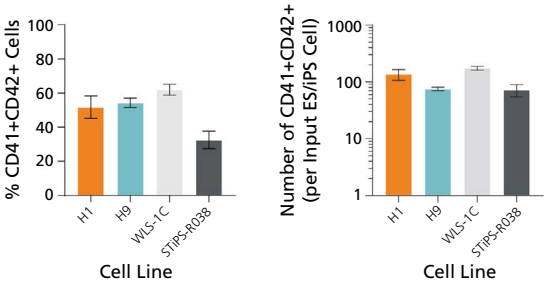


Figure 22. hESC- and hiPSC-Derived Hematopoietic Progenitor Cells Efficiently Expand and Differentiate to CD41a+CD42b+ Megakaryocytes

Frequencies and numbers of CD41a+CD42b+ MKs per input cell for two hESC lines (H1 and H9) and two hiPSC lines (WLS-1C and STiPS-R038). The average frequency of viable CD41a+CD42b+ cells on day 17 ranged between 56% and 77%. The average yield of CD41a+CD42b+ MKs generated per input cell ranged between 223 and 425. Data are shown as mean ± SEM (n = 12 for H1, n = 29 for H9, n = 27 for WLS-1C, n = 12 for STiPS-R038).

Learn more at **www.stemcell.com/megakaryocyte-diff**

STEMdiff™ Erythroid Kit

STEMdiff™ Erythroid Kit is designed for the serum-free and feeder-free differentiation of hESCs and hiPSCs to erythroid cells expressing CD71 and CD235ab. This optimized two-dimensional and two-stage protocol supports the generation of high yields of erythroid cells in 24 days.

Differentiate hPSCs to erythroid cells (erythroblasts) expressing Glycophorin A and CD71. hESCs or hiPSCs are induced toward erythroid-biased hematopoietic progenitor cells and then further differentiated to erythroid progenitor cells (Day 10 - 24). Cells generated using the STEMdiff™ Erythroid Kit can be further matured into normoblasts and reticulocytes once moved to appropriate culture conditions for maturation.

Why Use the STEMdiff™ Erythroid Kit?

CONSISTENT. Differentiate to erythroblasts in two stages, without feeders or serum.

HIGH YIELD. Expand hematopoietic progenitor cells by > 200-fold during erythroid differentiation, with > 70% yield for CD71+GlyA+ erythroblasts.

VERSATILE. hESC- and hiPSC-derived erythroblasts can be matured further using StemSpan™ SFEM II medium with appropriate supplements.

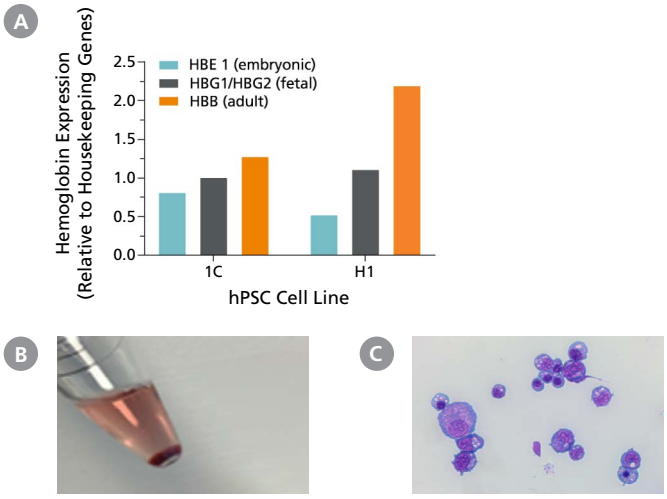


Figure 23. hESC- and hiPSC-Derived Erythroid Cells Are Hemoglobinized and Display Typical Erythroid Morphology

(A) Erythroid cells generated with the STEMdiff™ Erythroid Kit express a mix of primitive (embryonic) and definitive (fetal, adult) hemoglobin. Shown are the results of qPCR analysis for globin gene expression after 24 days of culture. (B) A picture of the cell pellet shows that cells produced in culture are hemoglobinized. (C) Cells display typical basophilic erythroblast morphology after 24 days of culture using the STEMdiff™ Erythroid Kit (40X magnification; May-Grunwald Giemsa stain).

Learn more at **www.stemcell.com/erythro-diff**

Recombinant Cytokines

Recombinant Human Cytokines

Cytokine	Catalog #	Unit Size (µg)
bFGF*	78003	50
BMP-2*	78004	50
BMP-4	78211	20
EGF*	78006	500
EPO	78007	50
EPO (HEK293-expressed)	100-1716	100
	100-1717	1000
FGF-4	78103	25
FGF-7 (KGF)*	78046	10
FGF-8B*	78008.1	50
FGF-10 (KGF-2)*	78037	10
FGF-18	78041	10
Flt3/Flk-2 Ligand*	78009	100
Flt3/Flk-2 Ligand (CHO-expressed)	100-1710	50
	100-1711	1000
G-CSF*	78012	100
GM-CSF (CHO-expressed)	78190	10
GM-CSF (E. coli-expressed)	78015	100
IFN-γ*	78020	100
IGF-I*	78022	500
IGF-II	78023	50
IL-1β*	78034	100
IL-2 (CHO-expressed)*	78036	50
IL-2 (E. coli-expressed)*	78220	10
IL-3 (CHO-expressed)*	78194	10
IL-3 (E. coli-expressed)*	78040	100
IL-4*	78045	100
IL-5	78048.1	10
IL-6	78050	100
IL-6Rα	78083.1	50
IL-7*	78053	100
IL-10*	78024	50
IL-11	78025	100
IL-12	78027	25
IL-13	78029	100
IL-15*	78031	100
IL-21*	78082	10
IL-31	78216	25
M-CSF*	78057	100
MIP-1β (CCL4)	78090	5
Oncostatin M*	78094	10
PDGF-AB	78096	10
PDGF-BB*	78097	10
PDGF-CC	78168	10
PDGF-DD	78222	25
R-Spondin-1	78213	25
SCF*	78062	100
SCF (P. pastoris-expressed)	100-1712	50
	100-1713	1000
TGF-β1	78067	5
TNF-α*	78068	50
TPO	78210	25
VEGF-121	78127	10
VEGF-165*	78073	50

**Animal Component-Free (ACF) version available.*

Recombinant Mouse Cytokines

Cytokine	Catalog #	Unit Size (µg)
BMP-2, ACF	78135	10
EGF	78016	10
FGF-8B, ACF	78204	25
FGF-21	78108.1	50
Flt3/Flk-2 Ligand	78011	100
G-CSF	78014	100
GM-CSF (CHO-expressed)	78206	10
IFN-γ	78021	100
IL-1β	78035	50
IL-2	78081	20
IL-3	78042	100
IL-4	78047	100
IL-5	78049.1	25
IL-6	78052	100
IL-7	78054	50
IL-10	78079	10
IL-11	78026	100
IL-12	78028.1	10
IL-13	78030.1	10
M-CSF	78059	100
MIP-1α (CCL3)	78089	10
MIP-1β (CCL4)	78091	10
PDGF-BB	78178	10
SCF	78064	100
SCF (P. pastoris-expressed)	100-1714	50
	100-1715	1000
SDF-1α (CXCL12)	78121	5
TNF-α	78069	100
TPO	78072	50
VEGF-164	78102	20

These high-quality cytokines ensure reproducibility across a variety of hematopoietic cell culture applications. Choose from a wide selection of cytokines, also available in other sizes, to incorporate into your research workflow. For a complete listing of cytokines, visit **www.stemcell.com/cytokines**.

MyeloCult™ Long-Term Culture Media

Detection of Primitive Progenitor Cells

Cell Sourcing & Isolation | Expansion & Differentiation | **Analysis**

Long-Term Culture Media

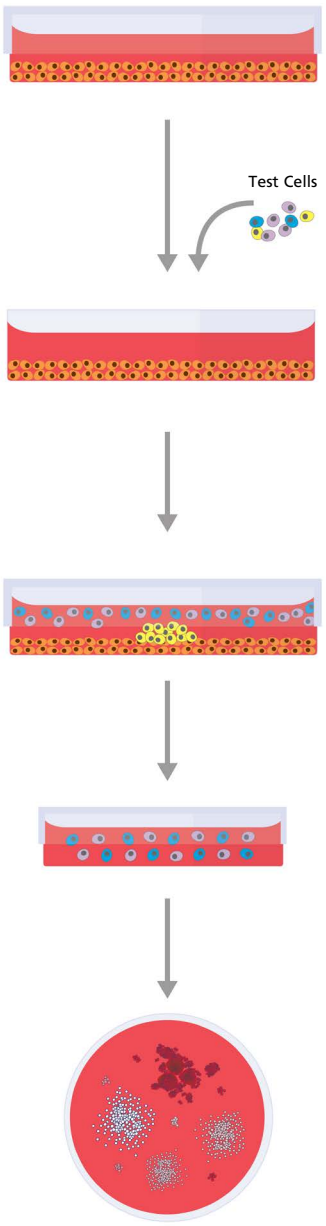
The long-term culture system for hematopoietic stem and progenitor cells (HSPCs), developed first for mouse marrow in the late 1970s, and then successfully adapted for human cells, establishes the essential cell types involved in hematopoiesis, in vitro.[16-17] When initiated with a relatively high density of bone marrow cells (> 10^6 cells/mL), long-term cultures are characterized by the formation of an adherent stromal layer of mesenchymal cells, including endothelial cells, fibroblasts, and adipocytes. Primitive hematopoietic cells associated with this stromal layer typically generate myeloid clonogenic progenitor cells and mature granulocytes for many weeks, provided that appropriate medium and supplements, incubation conditions, and feeding schedules are used.

MyeloCult™ is a unique long-term culture medium that promotes the formation of human or mouse primary stromal layers and allows the proliferation and differentiation of primitive hematopoietic progenitor cells.

Long-Term Culture-Initiating Cell Assay

The unique features of the long-term culture system have allowed the development of the long-term culture-initiating cell (LTC-IC) assay to detect and quantitate primitive hematopoietic cells, which share phenotypic and functional properties with mouse or human in vivo repopulating cells.[18-20] In human long-term cultures, colony-forming units (CFUs) detected after more than 5 weeks represent the progeny of LTC-ICs, as CFUs present in the input cell suspension will have undergone terminal differentiation by this time.

Quantitation of LTC-ICs in a test cell suspension requires culturing the cells on a supportive feeder layer of irradiated marrow cells or suitable human or mouse fibroblast cell lines.[21-22] Limiting dilution analysis is used to determine the frequency of LTC-ICs, as well as the average number of CFUs produced per LTC-IC. Once the average number of CFUs per LTC-IC is established, the LTC-IC content of a sample can be determined by a bulk culture LTC-IC assay, provided that the same source of test cells (e.g. bone marrow, mobilized peripheral blood, or cord blood) is used and the assay conditions are identical. The LTC-IC content is then calculated by dividing the total output of CFUs by the average number of CFUs produced per LTC-IC.[18]



1

Establish Feeders

Human: suitable cell line (e.g. M2-10B4)
Mouse: irradiated marrow cells

= feeder cell

2

Add Known Number of Test Cells

= LTC-IC
= CFU
= mature cell

3

Maintain Cultures

Weekly half medium changes using **MyeloCult™ H5100**
Human: 3 - 5 weeks
Mouse: 3 - 4 weeks

4

Harvest and Plate

Harvest entire dish, including adherent and non-adherent cells, and plate appropriate aliquot in methylcellulose cultures.
Human: 14 - 16 days, using **MethoCult™ H4435 Enriched**
Mouse: 12 days, using **MethoCult™ GF M3434**

5

Count Total Number of Colonies

Calculate number of LTC-ICs in test cell suspension based on the average output of CFUs per LTC-IC, as determined by a limiting dilution assay.

Figure 24. Bulk Culture LTC-IC Assay Procedure

Published Applications

Quantitate Frequency of LTC-ICs and Study Their Phenotypic and Functional Properties. Experiments using MyeloCult™ and related products show that LTC-ICs are a heterogeneous population of cells that can differ in phenotype, cell cycling characteristics, and expansion potential.[24-29]

Facilitate Gene Transfer. Culturing primitive hematopoietic progenitor cells in MyeloCult™ facilitates retroviral gene transfer and expansion of these transfected cells.[30,31]

Expand Multi-Potential Hematopoietic Cells In Vitro. Human colony-forming units (CFUs) and LTC-ICs have been expanded using MyeloCult™ in stirred suspension cultures.[32,33] Mouse totipotent hematopoietic cells expanded in static long-term cultures with MyeloCult™ can sustain lymphomyelopoiesis in irradiated recipients.[30]

Evaluate Factors Regulating Myelopoiesis. The role of stroma-derived factors (positive and/or negative regulators and adhesion molecules) in the regulation of myelopoiesis can be evaluated in long-term cultures using MyeloCult™.[34-37]

Examine Differences Between Normal and Malignant Cells. MyeloCult™ has been used to culture LTC-ICs from patients with chronic myeloid leukemia, acute myeloid leukemia, and aplastic anemia.[38-42]

Study Differentiation of CD34+ Cells into Natural Killer (NK) Cells. In the presence of IL-2 and IL-7, or SCF and IL-15, a subpopulation of CD34+ cord blood cells can be induced to differentiate into NK cells when cultured in MyeloCult™.[43]

Culture NK Cell Lines. MyeloCult™ has been used for the culture of the human NK-92 cell line.[44]

MyeloCult™ Media for Initiation and Maintenance of Myeloid Long-Term Cultures

Product Name	Catalog #	Size	Description
MyeloCult™ H5100	05150	500 mL	Myeloid long-term cultures of human hematopoietic progenitor cells and stromal feeder layers

MethoCult™ Media to Evaluate the Number of CFU per LTC-IC

Product Name	Catalog #	Size	Description
MethoCult™ H4435 Enriched	04435 04445	100 mL 24 x 3 mL	Detection of human colonies derived from LTC-ICs
MethoCult™ GF M3434	03434 03444	100 mL 24 x 3 mL	Detection of mouse colonies derived from LTC-ICs

Support Products

Product Name	Catalog #	Size	Description
Hydrocortisone	07904	100 mg	Supplementation of MyeloCult™ media to a final concentration of 10^{-6} M. Suitable for supplementing both human (Catalog #05150) and mouse (Catalog #05350) MyeloCult™ media for LTC-ICs assays.
L-Calc™ software	28600	N/A	Limiting dilution analyses for determination of frequencies and other applications NOTE: Free download of L-Calc™ Software is available at **www.stemcell.com/l-calc-software.html**

MethoCult™ Media

For Performing Colony-Forming Unit Assays

The colony-forming unit (CFU) assay is an in vitro functional assay for counting multipotential and lineage-committed hematopoietic progenitor cells (HPCs) in bone marrow, blood, and other hematopoietic tissues. MethoCult™ is a line of methylcellulose-based media formulated to promote optimal growth and differentiation of hematopoietic progenitor cells from various species (humans, non-human primates, mice, rats, and dogs). MethoCult™ is widely recognized as the "Gold Standard" for detection and quantification of hematopoietic progenitor cells in the CFU assay.

Key features of the CFU assay:

- Individual HPCs proliferate and differentiate to produce colonies of mature blood cells during culture in MethoCult™ medium containing growth factors and supplements.
- Progenitor cells of different lineages and stages of maturation produce colonies that differ in their size, morphology, and cellular composition.
- Each colony is derived from a single progenitor cell or CFU. The number of colonies provides a measure of the number of viable and functional CFUs in the cell sample being tested (i.e. 1 colony = 1 CFU).
- Enumerates all classes of myeloid and/or erythroid progenitor cells: erythroid (BFU-E and CFU-E), granulocyte-macrophage (CFU-GM, CFU-G, and CFU-M), and multi-potential progenitor cells (CFU-GEMM).

Why Use MethoCult™?

ROBUST. Ensure consistency and reproducibility in your experiments with standardized and rigorously tested media.

CONVENIENT. Identify and count total CFUs, erythroid (CFU-E and BFU-E), granulocyte/macrophage (CFU-GM, CFU-G, and CFU-M), and multi-lineage (CFU-GEMM) progenitor cells with ready-to-use formulations.

FLEXIBLE. Customize your cell-culture conditions by adding components of choice to suit your specific research needs.

BFU-E: burst-forming unit – erythroid; CFU-E: colony-forming unit – erythroid; CFU-GM: colony-forming unit – granulocyte/macrophage; CFU-G: colony-forming unit – granulocyte; CFU-M: colony-forming unit – macrophage; CFU-GEMM: colony-forming unit – granulocyte, erythrocyte, macrophage, megakaryocyte.

Applications

- Quantitation and characterization of human hematopoietic progenitor cells from cord blood, mobilized peripheral blood, and bone marrow[45]
- Quantitation and characterization of hematopoietic progenitor cells from mouse bone marrow and other cell samples
- Quantitation of primitive hematopoietic progenitor cells from human and mouse long-term culture-initiating cell (LTC-IC) assays[19,21]
- Evaluation of hematopoietic cell differentiation from human pluripotent stem cells (hPSCs)
- Quality control of cryopreservation, cell processing, and ex vivo manipulation procedures[46-54]
- Support of patient diagnosis, prognosis, and treatment in a clinical hematology lab[55-60]
- Support of the evaluation of donor samples, including cord blood, for stem cell transplants[61-66]
- Study of the effects of cytokines, growth factors, hormones, or mimetics on hematopoietic progenitor cells[67-71]
- Toxicity testing or drug screening assays[72-75]
- Optimization of gene transfer protocols and performing replating assays to study myeloid progenitor cell proliferation and self-renewal following genetic manipulation[76]
- Quantitation of hematopoietic progenitor cells following ex vivo expansion[27,68]

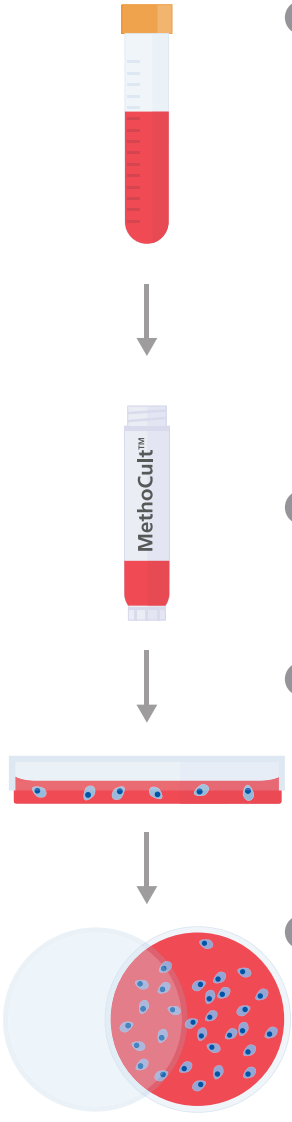
MethoCult™ H4034 Optimum Media in 24 x 3 mL and 100 mL Formats

The Colony Forming Unit (CFU) Assay

CFU Assay Workflow

Two of the most commonly performed assays for measuring the functionality of HSPCs are the in vivo mouse model engraftment study and the in vitro colony-forming unit (CFU) assay. Routine in vivo studies for potency assays are time-consuming, expensive, and difficult to validate. The CFU assay is significantly faster and more cost-effective compared to the in vivo mouse engraftment assay and has been shown to correlate well with in vivo engraftment in patients[62, 65-66]; it is thus gaining popularity as the method of choice for evaluating the quality and potency of HSPCs used in cell therapy. There are currently no specific recommendations from regulatory agencies regarding the potency testing of hematopoietic cell therapy products. However, several cord blood bank accrediting bodies (including AABB and FACT) now require pre- and post-processing CFU data from cord blood collection as evidence of sample stability and to confirm the minimal loss of HSPC functionality during processing and cryopreservation. These requirements highlight the growing importance of incorporating the CFU assay in clinical workflows.

How Does the CFU Assay Work?



1 Prepare Cells

Process cells by either:

- ammonium chloride lysis
- immunomagnetic depletion of red blood cells with ErythroClear™ (cord blood only)
- density sedimentation with HetaSep™ and SepMate™
- progenitor cell enrichment with EasySep™, RosetteSep™, or FACS (e.g. CD34+, KIT+, or SCA1+)

Wash cells (e.g. in Iscove's MDM plus 2% FBS), then count and adjust cell concentration.

2 Add Cells to MethoCult™

Add cells to MethoCult™ and vortex.

3 Plate and Incubate

Dispense cells into 35 mm culture dishes or SmartDish™ using a syringe and blunt-end needle.

Incubate human cells for 7-14 days, or mouse cells for 7-12 days, in a humidified incubator at 37°C and 5% CO_2.

4 Count Colonies

Count and evaluate colony types manually, using an inverted microscope and STEMgrid™-6 scoring grid, or automatically, using STEMvision™.

Note: Individual colonies may be plucked for routine staining, PCR, or cytogenetic analysis.

Figure 25. Colony-Forming Unit (CFU) Assay Procedure

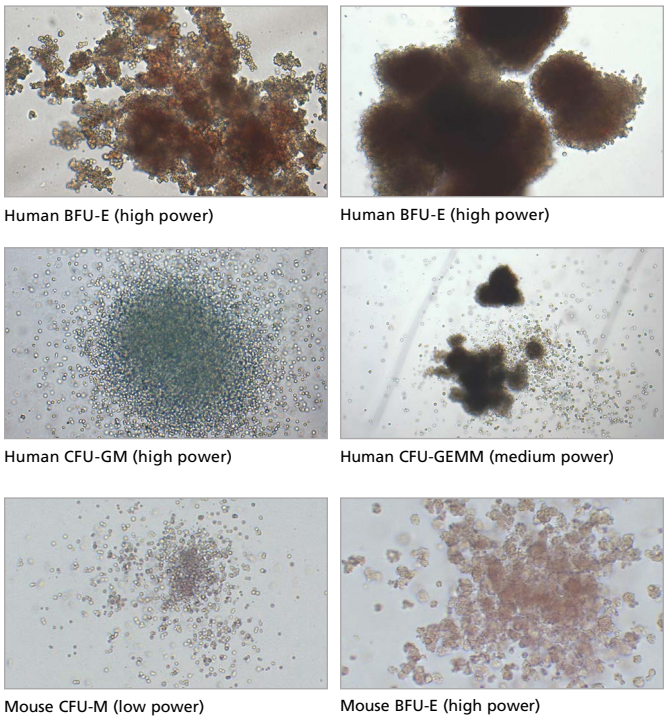


Figure 26. Images of Human and Mouse Colonies Visualized on an Inverted Microscope

On-Demand Training

Access Free Instruction on CFU Set Up, Colony Identification, and Scoring

www.stemcell.com/hsc-training

MethoCult™ Media for Human Cells

MethoCult™ Product	Catalog #	Unit Size	Components					Applications
			MC	FBS	BSA	Insulin + Transferrin	Growth Factors	
H4034 Optimum (GF H4034)	04034 04044	100 mL 24 x 3 mL	•	•	•		rhSCF, rhIL-3, rhG-CSF, rhEPO, rhGM-CSF	• Detection of CFU-E, BFU-E, CFU-GM, CFU-GEMM in BM, MPB, PB, and CB • Compatible with STEMvision™
H4035 Optimum Without EPO (GF H4035)	04035 04045	100 mL 24 x 3 mL	•	•	•		rhSCF, rhIL-3 , rhG-CSF, rhGM-CSF; no rhEPO	• Detection of CFU-GM in BM, MPB, PB, and CB • Compatible with STEMvision™
H4434 Classic (GF H4434)	04434 04444	100 mL 24 x 3 mL	•	•	•		rhSCF, rhIL-3, rhEPO, rhGM-CSF	Detection of CFU-E, BFU-E, CFU-GM, CFU-GEMM in BM, MPB, PB, and CB
H4534 Classic Without EPO (GF H4534)	04534 04544	100 mL 24 x 3 mL	•	•	•		rhSCF, rhIL-3, rhGM-CSF; no rhEPO	Detection of CFU-GM in BM, MPB, PB, and CB
H4435 Enriched (GF+ H4435)	04435 04445	100 mL 24 x 3 mL	•	•	•		rhSCF, rhIL-3, rhIL-6, rhEPO, rhG-CSF, rhGM-CSF	• Detection of CFU-E, BFU-E, CFU-GM, CFU-GEMM in BM, MPB, PB, and CB • Recommended for CD34+-enriched cells and cells isolated by other purification methods
H4535 Enriched Without EPO (GF+ H4535)	04535 04545	100 mL 24 x 3 mL	•	•	•		rhSCF, rhIL-3, rhIL-6, rhG-CSF, rhGM-CSF; no rhEPO	• Detection of CFU-GM in BM, MPB, PB, and CB • Recommended for CD34+-enriched cells and cells isolated by other purification methods
SF H4436	04436	100 mL	•		•	•	rhSCF, rhIL-3, rhIL-6, rhEPO, rhG-CSF, rhGM-CSF	Detection of CFU-E, BFU-E, CFU-GM, CFU-GEMM in BM, MPB, PB, and CB where a medium of defined composition is required
SF H4536	04536	100 mL	•		•	•	rhSCF, rhIL-3, rhIL-6, rhG-CSF, rhGM-CSF; no rhEPO	Detection of CFU-GM in BM, MPB, PB, and CB where a medium of defined composition is required
SF H4636	04636	100 mL	•		•	•	rhSCF, rhIL-3, rhG-CSF, rhGM-CSF, rhEPO	• Detection of CFU-E, BFU-E, CFU-GM, and CFU-GEMM • Recommended for the culture of human hPSC-derived hematopoietic progenitor cells in defined serum-free conditions • Use in CFU assays of human primary hematopoietic progenitor cells isolated from BM, MPB, PB, and CB
SFH4636 Without EPO	100-0945	100 mL	•		•	•	rhSCF, rhIL-3, rhG-CSF, rhGM-CSF; no rhEPO	• Detection of CFU-GM • Use in CFU assays of human primary hematopoietic progenitor cells isolated from BM, MPB, PB, and CB
Express	04437 04447	100 mL 24 x 3 mL	•	•	•		rhEPO and other cytokines	• Rapid CFU assays of human CB cells. Total colonies can be counted as early as 7 days after plating, without identification of colony type. • If cultures are maintained for 14 - 16 days, BFU-E, CFU-GM, CFU-G, CFU-M, and CFU-GEMM colonies can be counted. • Compatible with STEMvision™
H4431	04431	100 mL	•	•	•		Agar-LCM, rhEPO	• Detection of CFU-E, BFU-E, CFU-GM, CFU-GEMM in BM and PB • Suitable as a control medium for the detection of "EPO-independent" erythroid progenitor cells using MethoCult™ H4531
H4531	04531	100 mL	•	•	•		Agar-LCM; no rhEPO	• Detection of CFU-GM in BM and PB • Suitable for detection of "EPO-independent" erythroid progenitor cells
H4330	04330	90 mL	•	•	•		contains serum, rhEPO, no other cytokines	Allows researchers to add cytokines of their choice for applications including: • Drug toxicity testing in vitro • Detection of specific hematopoietic progenitor cells • Investigation of the action of novel factors • Hematopoietic colony assays in other species • Detection of genetically modified hematopoietic progenitor cells • Cloning and selection of non-adherent cell lines
H4230	04230	80 mL	•	•	•		contains serum, no cytokines	
SF H4236	04236	80 mL	•		•	•	serum-free, contains serum substitute, no cytokines	
H4100	04100	40 mL	•				base methylcellulose, no serum, serum substitutes or cytokines	

*For a full listing of products for analysis of HSPCs, visit **www.stemcell.com/HSPCworkflow**, under the "Analysis" tab.*

CFU-E: colony-forming unit – erythroid; BFU-E: burst-forming unit – erythroid; CFU-GM: colony-forming unit – granulocyte/macrophage; CFU-GEMM: colony-forming unit – granulocyte, erythrocyte, macrophage, megakaryocyte; BM: bone marrow; MPB: mobilized peripheral blood; PB: mobilized peripheral blood; CB: cord blood; hPSC: human pluripotent stem cell; EPO: erythropoietin; MC: methylcellulose; FBS: fetal bovine serum; BSA: bovine serum albumin.

MethoCult™ Media for Mouse Cells

MethoCult™ Product	Catalog #	Unit Size	Components					Applications
			MC	FBS	BSA	Insulin + Transferrin	Growth Factors	
GF M3434	03434 03444	100 mL 24 x 3 mL	•	•	•	•	rmSCF, rmIL-3, rhIL-6, rhEPO	• Assays of mouse hematopoietic progenitor cells (BFU-E, CFU-GM, CFU-G, CFU-M, CFU-GEMM) in BM, PB, spleen, and fetal liver • Compatible with STEMvision™
GF M3534	03534	100 mL	•	•	•	•	rmSCF, rmIL-3, rhIL-6; no rhEPO	• Assays of mouse hematopoietic progenitor cells (CFU-GM, CFU-G, CFU-M) in BM, PB, spleen, and fetal liver • Compatible with STEMvision™
M3630	03630	100 mL	•	•			rhIL-7	Assays of mouse pre-B clonogenic progenitor cells from BM and Whitlock-Witte long-term cultures
SF M3236	03236	80 mL	•		•	•	no cytokines	Assays of mouse hematopoietic progenitor cells from BM, PB, spleen, and fetal liver where a medium of defined composition is required
SF M3436	03436	100 mL	•		•	•	rhEPO and other cytokines	• Assays of erythroid progenitor cell (BFU-E) derived colonies from BM and other tissues • Compatible with STEMvision™
M3334	03334	90 mL	•	•	•	•	contains serum, rhEPO, no additional cytokines	Detection of CFU-E and mature BFU-E from mouse BM, spleen, and fetal liver
M3234	03234	80 mL	•	•	•	•	contains serum, no cytokines	Base medium for CFU assays, allowing addition of growth factors of choice
M3231	03231	80 mL	•	•	•		contains serum, no insulin or transferrin, no cytokines	• Base medium for CFU assays, allowing addition of growth factors of choice • Cloning of cell lines
M3134	03134	40 mL	•				base methylcellulose, no serum, serum substitutes or cytokines	Base medium for CFU assays, allowing researchers flexibility in addition of desired components

MC: methylcellulose; FBS: fetal bovine serum; BSA: bovine serum albumin; BM: bone marrow; MPB: mobilized peripheral blood; CB: cord blood; LCM: leukocyte-conditioned medium; BFU-E: burst-forming unit – erythroid; CFU-E: colony-forming unit – erythroid; CFU-G: colony-forming unit – granulocyte; CFU-GM: colony-forming unit – granulocyte/macrophage; CFU-M: colony-forming unit – macrophage; CFU-GEMM: colony-forming unit – granulocyte, erythrocyte, macrophage, megakaryocyte.

STEMvision™

Automated and Standardized Counting of CFU Assays

STEMvision™ is an instrument and software system designed for imaging and counting hematopoietic colonies in the colony-forming unit (CFU) assay.

Instead of manually counting colonies, users simply load a SmartDish™ (Catalog #27370) plate containing cells plated in MethoCult™ medium into STEMvision™. Digital images of individual 35 mm wells of the 6-well SmartDish™ plate containing human or mouse cells are acquired in approximately 1 minute or 30 seconds, respectively. Analysis requires approximately 1 minute for assays of human cells and 30 seconds for assays of mouse cells, and can be performed at a later time or overnight.

For human cultures, three STEMvision™ software packages have been designed for scoring and counting colonies produced by erythroid, myeloid, and multi-potential progenitor cells (BFU-E, CFU-E, CFU-GM/G/M, and CFU-GEMM) in 14-day CFU assays of cells isolated from cord blood (CB), bone marrow, and mobilized peripheral blood, cultured in MethoCult™ Optimum. A fourth software package is available to count the total number of colonies (without classification of CFU subtypes) in 7-day CFU assays of CB cells in MethoCult™ Express.

For mouse bone marrow cultures, three STEMvision™ software packages have been designed to count the total number of colonies produced by all combined myeloid and erythroid progenitor cells (BFU-E and CFU-GM/G/M) in MethoCult™ GF M3434, all myeloid progenitor cells (CFU-GM/G/M) in MethoCult™ GF M3534, and all erythroid progenitor cells (BFU-E) in MethoCult™ SF M3436.

SYSTEM IS SUPPLIED WITH:

- STEMvision™ instrument
- Computer and monitor
- Software for image acquisition, analysis, and review

REQUIRED REAGENTS:

- SmartDish™ meniscus-free cultureware
- MethoCult™ medium
- Red blood cell depletion reagent

CAPACITY:

- One 6-well SmartDish™ per run

STEMvision™ Instrument

Visit **www.STEMvision.com** for more information.

Why Use STEMvision™?

ACCURACY. Achieve faster and more accurate colony counts than with manual counting.

STANDARDIZE. Eliminate the subjectivity associated with manual colony counting.

CONVENIENT. Easily save and share your results. Images are automatically stored for record keeping.

Software for Human Assays

PRODUCT:	STEMvision™ Human Cord Blood 7-Day CFU Analysis Package
CATALOG #:	22001
PRODUCT:	STEMvision™ Human Cord Blood 14-Day CFU Analysis Package
CATALOG #:	22005
PRODUCT:	STEMvision™ Human Bone Marrow 14-Day CFU Analysis Package
CATALOG #:	22006
PRODUCT:	STEMvision™ Human Mobilized Peripheral Blood 14-Day CFU Analysis Package
CATALOG #:	22007

Software for Mouse Assays

PRODUCT:	STEMvision™ Mouse Total CFU Analysis Package
CATALOG #:	22008
PRODUCT:	STEMvision™ Mouse Myeloid CFU Analysis Package
CATALOG #:	22009
PRODUCT:	STEMvision™ Mouse Erythroid CFU Analysis Package
CATALOG #:	22011

Software for Quality Management

PRODUCT:	STEMvision™ 21 CFR Part 11 Compliance Software Add-On
CATALOG #:	500-0110

Human Cord Blood CFU Assays

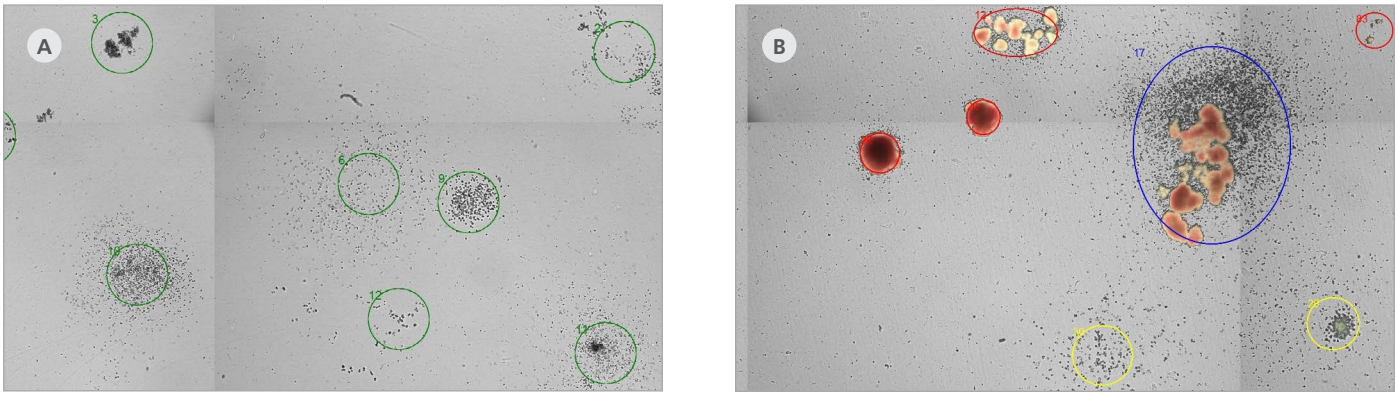


Figure 27. Representative STEMvision™ Images Showing Colonies Derived from Human Cord Blood Progenitor Cells After 7 Days of Culture in MethoCult™ Express or After 14 Days of Culture in MethoCult™ Optimum

The images have been analyzed with the STEMvision™ Human Cord Blood (A) 7-Day (Catalog #22001) and (B) 14-Day (Catalog #22005) Analysis Packages. Green circles identify individual colonies in the 7-day CB CFU assay that counts the total number of CFUs. Red circles identify erythroid colonies (produced by BFU-E), yellow circles identify myeloid colonies (produced by CFU-GM, CFU-G or CFU-M), and blue circles identify mixed colonies (produced by CFU-GEMM) in the 14-day CB CFU assay. Erythroid and mixed colonies that contain hemoglobinized cells are shown in true red color.

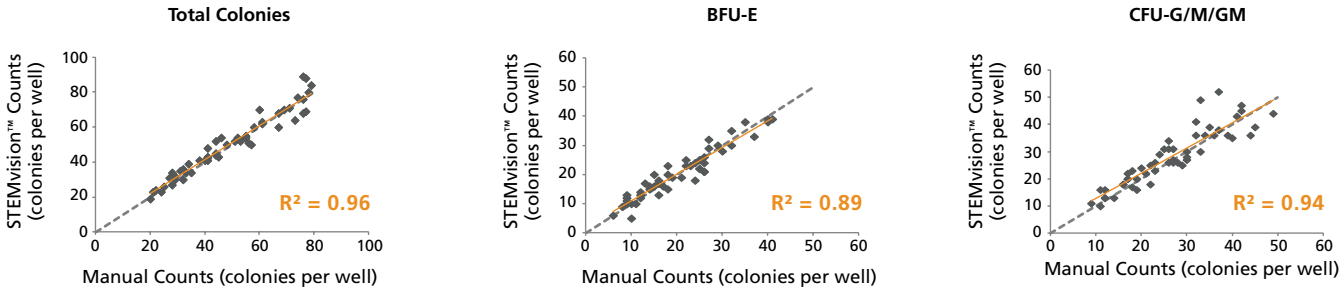


Figure 28. STEMvision™ Automated Counts of Total, Erythroid (BFU-E), and Myeloid (CFU-G/M/GM) Colonies Are Highly Correlated to Manual Counts of 14-Day CB CFU Assays

Cryopreserved CB samples were thawed, plated in MethoCult™ Optimum, cultured for 14 days, and scored both manually using an inverted microscope and automatically using STEMvision™. The results show a strong correlation between automated counts using STEMvision™ and manual counts. Gray dashed lines represent a perfect linear correlation between manual and automated counts. Orange solid lines represent the actual linear correlation between manual and automated counts (n = 130 CFU assays).

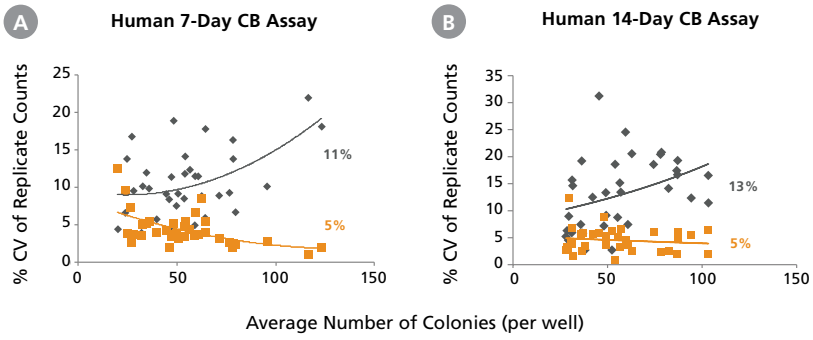


Figure 29. STEMvision™ Automated Colony Counting of 7-Day and 14-Day CB CFU Assays Is More Reproducible Than Manual Counting

The coefficients of variation (CV) for total colony counts in (A) 7-day and (B) 14-day CFU assays of CB cells were determined by counting the same culture wells manually by 3 to 5 different people (gray diamonds) and automatically using 3 to 5 separate STEMvision™ instruments (orange squares). The average CVs for 7-day and 14-day total colony counts produced manually were 11% and 13%, respectively. CVs for 7-day and 14-day colony counts produced by STEMvision™ were 5%.

Mouse Bone Marrow CFU Assays

MethoCult™ GF M3434

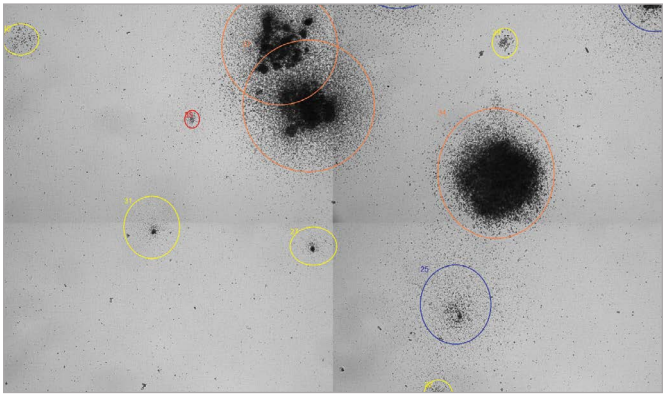


Figure 30. Representative STEMvision™ Images Showing Colonies Derived from Mouse Bone Marrow Progenitor Cells After 12 Days of Culture in MethoCult™ GF M3434 Medium

Images of mouse bone marrow cells cultured in MethoCult™ GF M3434 medium were acquired using STEMvision™. The Total CFU Analysis Package (Catalog #22008) was used to analyze the image. Red circles identify the smallest colonies; size class 1, yellow circles; size class 2, blue circles; size class 3, and orange circles identify the largest colonies; size class 4.

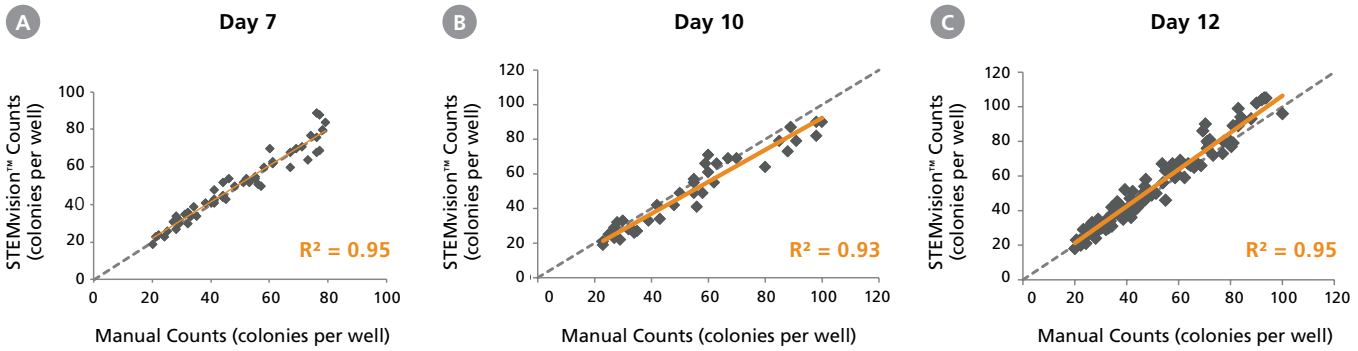


Figure 31. STEMvision™ Automated Total Colony Counts (Myeloid Plus Erythroid) on Days 7, 10, and 12 Are Highly Correlated to Manual Counts of CFU Assays Using Mouse Bone Marrow Cells

Bone marrow cells were plated in MethoCult™ GF M3434. Colonies were counted on days (A) 7, (B) 10, and (C) 12 both manually using an inverted microscope, and automatically using STEMvision™ equipped with the Mouse Total CFU Analysis Package (Catalog #22008). We recommend counting CFU assays of mouse progenitor cells plated in M3434 between 7 and 12 days. Gray dashed lines represent a theoretical perfect linear correlation between manual and automated counts. Orange solid lines represent the actual linear correlation between manual and automated counts. Coefficients of determination (R^2) that describe each data set (n = 104 CFU assays in A, n = 38 in B, and n = 99 in C) are shown in orange.

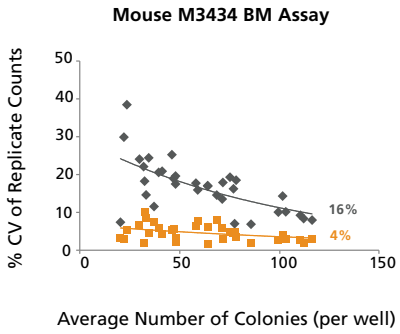


Figure 32. STEMvision™ Automated Colony Counting of CFU Assays with Mouse Cells Is More Reproducible Than Manual Counting

The coefficients of variation (CVs) for total colony counts in CFU assays of mouse BM in MethoCult™ GF M3434 were determined by counting the same culture dishes manually by 3 to 5 different people (grey diamonds) and automatically using 3 to 5 separate STEMvision™ instruments (orange squares). The average CV for total colony counts produced manually was 16%. The average CV for colony counts produced by STEMvision™ was 4%.

SmartDish™ and STEMgrid™-6

Meniscus-Free Cultureware for More Accurate Counting of Hematopoietic Colonies

When the CFU assay is performed using traditional cultureware, the medium forms a meniscus where it meets the dish. The greater medium depth in the meniscus results in a greater proportion of colonies forming around the periphery (Figure 33A) where optical distortion can make it challenging to identify colonies (Figures 34A and 34C). This can reduce the accuracy of colony counting (i.e. result in undercounting of CFUs).

SmartDish™ 6-well culture plates have been designed to enable accurate and reproducible colony counting by preventing the formation of a meniscus. This allows for more uniform distribution of culture medium (Figure 33B), which results in a more even distribution of colonies throughout the entire 35 mm well. Additionally, the absence of the meniscus reduces optical distortion so that colonies present at the edge of the dish can be more easily counted (Figures 34B and 34D).

SmartDish™ has been designed to work with standard inverted microscopes when performing manual colony counts (Figure 34B), as well as with the STEMvision™ instrument for automated counting of hematopoietic CFU assays (Figure 34D). For manual counting of hematopoietic CFU assays, SmartDish™ is used with STEMgrid™-6, a detachable counting grid that facilitates navigation throughout the culture and also divides it into four quadrants for partial counting if desired.

SmartDish™ Meniscus-Free Cultureware

PRODUCT:	SmartDish™ (6-well plates)	
CATALOG #:	27370	(5/pack)
	27371	(50/pack)

RECOMMENDED FOR:

- Easier and more accurate colony counting
- Automated colony counting with STEMvision™ (required)

STEMgrid™-6 Counting Grid

PRODUCT:	STEMgrid™-6	
CATALOG #:	27000	(1/pack)

RECOMMENDED FOR:

- Manual colony counting in SmartDish™ cultureware under an inverted microscope

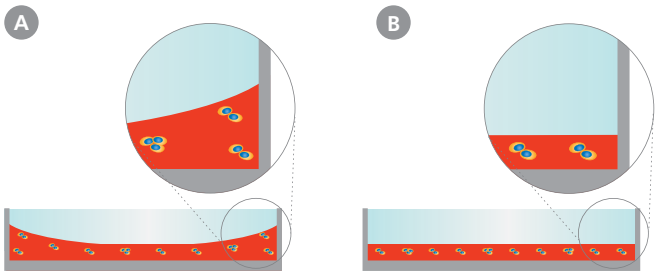
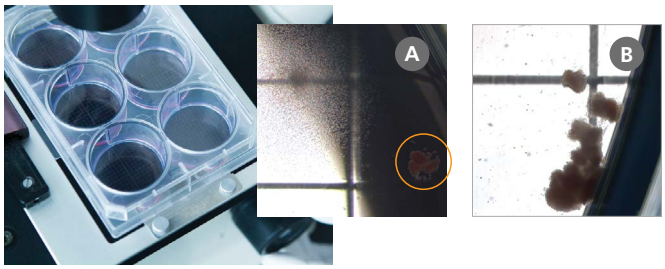


Figure 33. Schematic Illustration of Medium and Colony Distribution in Standard and SmartDish™ Cultureware

(A) The formation of a meniscus in standard cultureware results in more colonies forming around the dish edge where the culture medium is deeper. (B) The absence of a meniscus in SmartDish™ cultureware ensures more uniform distribution of culture medium and colonies throughout the entire well.

Manual Counting of Hematopoietic CFU Assays



Automated Counting of Hematopoietic CFU Assays

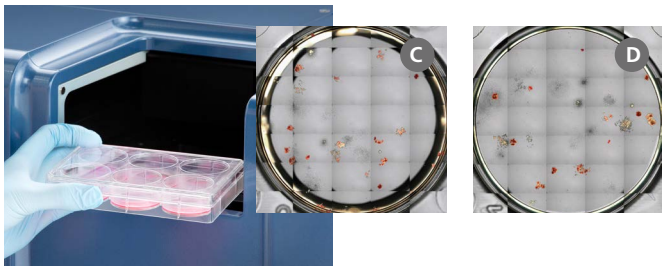


Figure 34. 14-Day Human Cord Blood CFU Assays Performed in Standard Non-Treated and SmartDish™ 6-Well Culture Plates

Shown are representative STEMvision™ images of 35 mm wells from either a (A, C) non-treated 6-well plate or (B, D) SmartDish™. The formation of a meniscus in standard cultureware causes more colonies to form around the periphery of the dish where the culture medium is deeper (A, C). Optical distortion obscures these colonies and makes them more difficult to count. Colonies are easier to count at the edge of the SmartDish™, which has been treated to eliminate the meniscus, allowing a more equal distribution of colonies (B, D).

MegaCult™ Collagen-Based Media

For the Detection of Megakaryocyte Progenitor Cells and for Permanent Records of Hematopoietic Progenitor Assays

Why Use Collagen-Based Media?

Cultures in collagen-based media can be dehydrated and fixed and have been shown to support the proliferation of hematopoietic progenitor cells.[77] This is beneficial when quantifying and detecting megakaryocyte colonies, which cannot be distinguished morphologically from macrophage colonies. Their identification requires the use of staining procedures to identify the expression of megakaryocyte-specific cell surface markers or enzymatic activity. Cellular and molecular analysis of cells cultured in methylcellulose-based media requires that the colonies be plucked and processed, which is time-consuming. In contrast, dehydrating and fixing a collagen-based culture prior to staining only requires about 30 minutes. Collagen-based cultures that have been dehydrated, fixed, and stained can be maintained as long-term records. This is in contrast to methylcellulose-based cultures that can only be maintained for approximately one week after the culture period.

Why Use MegaCult™?

CONVENIENT. Culture and stain your cells on one slide and evaluate immediately or store for examination at a later time.

OPTIMIZED. Quantify human megakaryocytic progenitor cells in serum-free conditions.

FLEXIBLE. Customize your cell-culture conditions by adding desired cytokines for the assay of human or mouse megakaryocytic progenitor cells.

For more information, visit **www.MegaCult.com**.

Human and Mouse MegaCult™ Media and Staining Kits

Product Name	Catalog #	Components	Applications
MegaCult™-C Complete Kit with Cytokines	04971	• Serum-free medium with recombinant cytokines (24 x 2 mL) • Collagen solution (35 mL) • CFU-Mk staining kit • Double chamber slides (48)	Detection and staining of human megakaryocyte progenitor cells in light density or CD34+-enriched BM, MPB, and CB cells
MegaCult™-C Complete Kit Without Cytokines	04970	• Serum-free medium without cytokines (24 x 1.7 mL) • Collagen solution (35 mL) • CFU-Mk staining kit • Double chamber slides (48)	Detection and staining of human megakaryocyte progenitor cells in light density or CD34+-enriched BM, MPB, and CB cells (requires addition of appropriate cytokines)
MegaCult™-C Collagen and Medium with Cytokines	04961	• Serum-free medium with recombinant cytokines (24 x 2 mL) • Collagen solution (35 mL)	Detection of human megakaryocyte progenitor cells in light density or CD34+-enriched BM, MPB, and CB cells
MegaCult™-C Collagen and Medium Without Cytokines	04960	• Serum-free medium without cytokines (24 x 1.7 mL) • Collagen solution (35 mL)	Detection of human or mouse megakaryocyte or other progenitor cells (requires addition of appropriate cytokines)
MegaCult™-C Collagen and Medium with Lipids	04974	• Serum-free medium with lipids, without cytokines (50 mL) • Collagen solution (35 mL)	Detection of human or mouse megakaryocyte or other progenitor cells (requires addition of appropriate cytokines)
MegaCult™-C Staining Kit for CFU-Mk	04962	• Anti-CD41 primary antibody • Anti-TNP control antibody • Biotin-conjugated secondary antibody • Alkaline phosphatase detection system • Human serum for dilutions and BSA for blocking • Evans Blue counterstain	Immunocytochemical staining for detection of human megakaryocytes and platelets in CFU-Mk and BFU-E/Mk grown in MegaCult™-C

CFU-Mk: colony-forming unit – megakaryocyte; BFU-E/Mk: burst-forming unit – erythroid/megakaryocyte; BM: bone marrow; MPB: mobilized peripheral blood; CB: cord blood.

Antibodies and ELISA Kits for HSPC Research

Analyze cells with antibodies that are verified to work with STEMCELL Technologies' cell isolation and cell culture reagents for select applications. These high-quality antibodies ensure consistent results for your downstream cell analysis, such as phenotyping and purity assessments of primitive cells and expanded progenitors.

For a complete product listing, including secondary antibodies and isotype controls, visit **www.stemcell.com/antibodies**. Alternatively, to view available ELISA kits for HSPC research, including the Erythropoietin (EPO) ELISA Kit (Catalog #01630), visit **www.stemcell.com/ELISA**.

Antibodies for Human Hematopoietic Cell Research

Antigen	Clone	Isotype	Conjugate	Catalog #
CD3	SK7	Mouse IgG_1, kappa	APC, FITC, PE, PerCP	60127
	UCHT1	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP, PerCP-Cy5.5	60011
CD11b	ICRF44	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60040
	M1/70	Rat IgG_{2b}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, Pacific Blue™, PE, PerCP-Cy5.5	60001
CD14	MoP9	Mouse IgG_{2b}, kappa	FITC	60124
	M5E2	Mouse IgG_{2a}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60004
CD16	3G8	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60041
CD19	HIB19	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60005
	6D5	Rat IgG_{2a}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, Pacific Blue™, PE	60006
CD20	2H7	Mouse IgG_{2b}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60008
CD34	8G12	Mouse IgG_1, kappa	APC, FITC, PE	60121
	563	Mouse IgG_1, kappa	PE	60119
	581	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60013
CD38	AT-1	Mouse IgG_1, kappa	FITC	60131
	HIT2	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60014
CD45	2D1	Mouse IgG_1, kappa	FITC	60123
	HI30	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, Pacific Blue™, PE, PerCP-Cy5.5	60018
CD45RA	HI100	Mouse IgG_{2b}, kappa	Unconjugated, APC-Cyanine7, Biotin, PE	100-0318
CD71 (Transferrin Receptor)	OKT9	Mouse IgG_1, kappa	Unconjugated, APC, Biotin, FITC, PE	60106
CD90 (Thy-1)	5E10	Mouse IgG_1, kappa	Unconjugated, APC, Biotin, FITC, PE, PerCP-Cy5.5	60045
CD105	43A3	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60039
CD117 (cKit)	104D2	Mouse IgG_1, kappa	Unconjugated, APC, Biotin, PE	60087
CD123 (IL-3Rα)	6H6	Mouse IgG_1, kappa	Unconjugated, APC, Biotin, FITC, PE, PerCP-Cy5.5	60110
CD235a (Glycophorin A)	2B7	Mouse IgG_1, kappa	FITC	60152
CD235ab (Glycophorin A/B)	HIR2	Mouse IgG_{2b}, kappa	Unconjugated, APC, Biotin, FITC, PE	60111

Antibodies for Mouse Hematopoietic Cell Research

Antigen	Clone	Isotype	Conjugate	Catalog #
CD3	17A2	Rat IgG_{2b}, kappa	Unconjugated, APC, FITC, PerCP-Cy5.5	100-1606
CD3e	145-2C11	Hamster (Armenian) IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60015
CD4	GK1.5	Rat IgG_{2b}, kappa	Unconjugated, APC, FITC, PE, PerCP-Cy5.5	100-1601
	RM4-5	Rat IgG_{2a}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60017
	RM4-4	Rat IgG_{2b}, kappa	APC, Biotin, FITC, PE	60029
CD8a	2.43	Rat IgG_{2b}, kappa	Unconjugated, APC, FITC	100-1633
	53-6.7	Rat IgG_{2a}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60023
CD11b	M1/70	Rat IgG_{2b}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, Pacific Blue™, PE, PerCP-Cy5.5	60001
CD19	6D5	Rat IgG_{2a}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, Pacific Blue™, PE	60006
CD43	R2/60	Rat IgM, kappa	Biotin	60042
CD45.1	A20	Mouse IgG_{2a}, kappa	APC, Biotin, FITC, PE	60117
CD45.2	104	Mouse IgG_{2a}, kappa	APC, Biotin, FITC, PE	60118
CD45	30-F11	Rat IgG_{2b}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60030
CD48 (SLAMF2)	HM48-1	Hamster (Armenian) IgG_1, lambda	APC	60162
CD45R (B220)	RA3-6B2	Rat IgG_{2a}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, Pacific Blue™, PE, PerCP-Cy5.5	60019
CD80 (B7-1)	16-10A1	Hamster (Armenian) IgG	APC, FITC, PE	100-1622
CD86 (B7-2)	GL-1	Rat IgG_{2a}, kappa	APC, FITC, PE	100-1625
CD117 (cKit)	2B8	Rat IgG_{2b}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60025
	ACK2	Rat IgG_{2b}, kappa	Unconjugated, Alexa Fluor® 488, APC, FITC, PE	60034
CD150 (SLAM)	TC15-12F12.2	Rat IgG_{2a}, lambda	Unconjugated, Alexa Fluor® 488, APC, Biotin, PE, PE-Cyanine7	60036
EPCR (CD201)	RMEPCR1560	Rat IgG_{2b}, kappa	Unconjugated, PE	60038
Gr-1 (Ly-6G/Ly-6C)	RB6-8C5	Rat IgG_{2b}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60028
Ly-6C	HK1.4	Rat IgG_{2c}, kappa	APC, PE, PerCP-Cy5.5	100-1636
Ly-6G	1A8	Rat IgG_{2a}, kappa	Unconjugated, APC, Biotin, FITC, Pacific Blue™, PE, PerCP-Cy5.5	60031
Sca1 (Ly-6A/E)	E13-161.7	Rat IgG_{2a}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60032
Rhesus	T3G6	Mouse IgG_1, kappa	Unconjugated	60132
	T4G6	Mouse IgG_1, kappa		60133
TCR Beta	H57-597	Hamster (Armenian) IgG	Unconjugated, APC, FITC, PE, PerCP-Cy5.5	100-1610
TER119	TER-119	Rat IgG_{2b}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60033

Detection Assays for Cord Blood Potency Testing

ALDH^{br} Assay Kit

The ALDH^{br} Assay Kit (Catalog #01711) is optimized for the identification and quantitation of viable CD34$^+$ cells that express high levels of the enzyme aldehyde dehydrogenase (ALDH) in human cord blood samples.

Product Information

Products	Catalog #	Components
ALDH^{br} Assay Kit*	01711	**ALDEFLUOR™ Kit** • ALDEFLUOR™ Reagent • ALDEFLUOR™ DEAB Reagent • ALDEFLUOR™ Assay Buffer • 2N HCl • DMSO **Viability Dye and Antibodies** • 7-AAD Viability Dye • APC CD34 Antibody (Clone 581) • PE CD45 Antibody (Clone HI30) • PE-Cyanine5 CD235ab (GlyAB) Antibody (Clone HIR2)"

**Suitable for labeling up to 6 x 10^7 cord blood cells*

For more information, including data and a full protocol, visit **www.stemcell.com/ALDHbrKit**.

Tissue Culture Reagents and Supplies

A variety of support products are available to accompany STEMCELL Technologies' complete array of cell separation and specialized media products. For more details and a full list of tissue culture reagents and supplies, visit **www.stemcell.com**.

Tissue Culture Media

Product Name	Catalog #	Unit Size
Agar Leukocyte Conditioned Medium	02300	25 mL
DMEM with 4500 mg/L D-glucose	36250	500 mL
DMEM with 1000 mg/L D-glucose	36253	500 mL
DMEM/F-12 with 15mM HEPES	36254	500 mL
Iscove's MDM (IMDM)	36150	500 mL
IMDM with 2% FBS	07700	100 mL
McCoy's 5A Medium	36350	500 mL
MEM Alpha with Nucleosides	36450	500 mL
MEM Alpha without Nucleosides	36453	500 mL
MEM with Earle's & NEAA	36550	500 mL
ReproTeSR™ (2-Component)	05926	500mL

Balanced Salt Solution

Product Name	Catalog #	Unit Size
D-PBS	37350	500 mL
D-PBS, 10X	37354	500 mL
D-PBS with 2% FBS	07905	500 mL
HBSS, Ca++ & Mg++ free	37250	500 mL
HBSS, Without Phenol Red	37150	500 mL

Supplements

Product Name	Catalog #	Unit Size
Heparin Solution	07980	2 mL
Hydrocortisone Powder	07904	100 mg
L-glutamine, 200 mM	07100	100 mL
MEM Non-Essential Amino Acids, 100X	07600	100 mL
Human Low-Density Lipoproteins (LDL)	02698	5 mg

Enzymes

Product Name	Catalog #	Unit Size
Collagenase Type I (0.25%)	07902	5 mL
DNase I, 1 mg/mL	07900	1 mL
Trypsin in Citrate Saline (0.25%)	07400	100 mL
Trypsin-EDTA (0.25%)	07901	500 mL
Trypsin-EDTA (0.05%)	07910	500 mL

Serum Substitutes

Product Name	Catalog #	Unit Size
10% Bovine Serum Albumin (BSA) in Iscove's MDM	09300	100 mL
BIT 9500 Serum Substitute	09500	100 mL

Miscellaneous Tissue Culture Reagents

Product Name	Catalog #	Unit Size
3% Acetic Acid with Methylene Blue	07060	100 mL
7-AAD (7-Aminoactinomycin D)	75001	500 tests
Ammonium Chloride Solution	07800 07850	100 mL 500 mL
DAPI (Hydrochloride)	75004	10 mg
Hydrocortisone	74142 74144	100 mg 1 g
Propidium Iodide	75002	10 mg
Trypan Blue	07050	100 mL

Tissue Culture Dishes & Slides

Product Name	Catalog #	Unit Size
Outer Dishes for CFU Assays		
Corning® 245 mm Square Dish, Non-Treated	38020	4/pack 16/case
Non-Adherent Culture Dishes *Recommended for CFU Assays using MethoCult™		
35 mm Diameter Dishes	27100 27150	10 Dishes 500 Dishes
SmartDish™	27370 27371	5/pack 50/pack
Other Dishes, Flasks, and Slides		
245 mm Square Dish, Tissue Culture-Treated	38039 100-0084	16 Plates 4 Plates
Corning® 60 mm Gridded Scoring Dish	38068 100-0085	500 Dishes 20 Dishes
Corning® Disposable Erlenmeyer Flask, 500 mL	38013	25 Flasks
Corning® Disposable Erlenmeyer Flask, 250 mL	38012	50 Flasks
Corning® Disposable Erlenmeyer Flask, 125 mL	38011	50 Flasks
Double Chamber Slide Kit	04963	48 double chamber slides, filter cards and spacers
Falcon® 24-Well Flat-Bottom Plate, Tissue Culture-Treated	38021	50 Plates
Falcon® 96-Well Flat-Bottom Microplate, Tissue	38022	50 Plates
Culture-Treated		
Falcon® Round-Bottom Tubes, 14 mL	38008	500 Tubes
Tissue Culture-Treated Dishes, 100 mm	38046 100-0082	500 Dishes 20 Dishes
Tissue Culture-Treated Dishes, 100 mm	27125 27127	10 Dishes 240 Dishes

Miscellaneous Tissue Culture Supplies

Product Name	Catalog #	Unit Size
3 cc Syringes	28240	100 Syringes/bag
Blunt-End Needles	28110 28120	100/pack 2000/case
Reversible Strainer, 37 µm	27215 27250	Small, 50/box Large, 25/box
Hypoxia Chamber	27310	1 chamber

Proficiency Testing Programs and Quality Control Kits

STEMCELL Technologies is committed to helping you get the most out of your experiments. Our standardization tools include proficiency testing programs, quality control kits, training courses, and instructional materials to improve your competency in performing hematopoietic colony-forming unit (CFU) assays.

Proficiency Testing Programs

Evaluate your ability to perform all steps of the CFU assay, from thawing samples to plating cells in methylcellulose medium, in our Proficiency Testing programs. Participants are provided with a cell sample, MethoCult™ medium, additional reagents and supplies, and detailed instructions required to perform the CFU assay. The results from all participants are analyzed and compared to the cohort mean (Figure 35) according to guidelines outlined in ISO 13528.

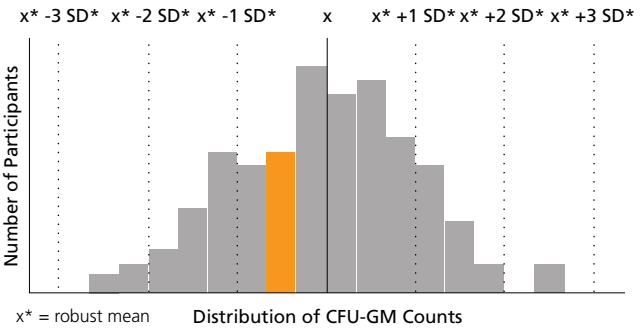


Figure 35. Example Participant Data

Proficiency Testing Supplies

Proficiency Testing Program		Catalog #
Frozen Human Bone Marrow (BM)	Spring Fall	100-0926 100-0928
Fresh Cord Blood (CBH)	Spring	00606
Frozen Cord Blood (CBZ)	Spring Fall	100-0950 100-0952

For information on upcoming program dates and how to register, visit **www.ProficiencyTesting.com**.

Quality Control Kits

STEMCELL Quality Control Kits are recommended for technologists in cell processing laboratories wanting to monitor their ability to reproducibly set up and score colonies in hematopoietic CFU assays. The use of a standardized medium and identical aliquots of cells from a single source minimizes potential variability associated with these components of the assay, allowing a more accurate assessment of an individual's technique. Each kit includes sufficient supplies to perform monthly CFU assays over a 1 year period.

Quality Control Kit Supplies

Quality Control Kit	Catalog #	Application
Human Bone Marrow (BM)	00650	Monitoring reproducibility in performing BM CFU assays
Human Cord Blood (CB)	00651	Monitoring reproducibility in performing CB CFU assays

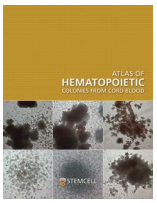
Training Courses and Instructional Materials

Our hematopoietic assay training courses are comprehensive and provide both theoretical and hands-on training. Our instructors share their knowledge and expertise to help overcome challenges in assay design, set up, and evaluation.

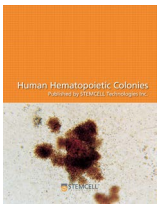
Live Training Course for Hematopoietic Progenitor Assays

Course Name	Catalog #	Course Description
Standardization of the Hematopoietic Progenitor Assay	00215	This 2-day course focuses on the standardization of the colony-forming unit (CFU) assay for human samples • Gain hands-on experience in the assay set-up, identification, and enumeration of hematopoietic progenitor cells using MethoCult™

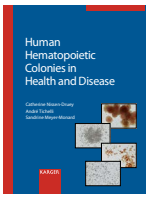
STEMCELL Technologies also offers customized training courses, which can be highly beneficial to address specific research challenges or to standardize technical processes. Custom training packages provide your team with personalized instruction from a technical specialist at your facility or at our training laboratory. For more information about courses or customized training, visit **www.stemcell.com/training** or contact **education@stemcell.com.**



Catalog #29940



Catalog #28700



Catalog #28760

Instructional Materials

Product Name	Catalog #	Description
Cord Blood Colony Atlas	29940	Detailed color images of hematopoietic colonies derived from human umbilical cord blood progenitor cells grown in methylcellulose-based media. Request a free hard copy at **www.stemcell.com/cbc-atlas**.
Human Hematopoietic Colony Atlas	28700	A guide for the process of identifying and evaluating colonies derived from human hematopoietic progenitor cells grown in methylcellulose-based media. Request a free hard copy at **www.stemcell.com/human-atlas**.
Human Hematopoietic Colonies in Health and Disease	28760	Colony-morphology from healthy individuals and patients with hematological disorders are illustrated in 230 detailed, color photographs.

Technical Manuals for Hematopoietic Progenitor Assays

Technical Manuals are available to support hematopoietic media products and assay systems. These manuals provide detailed reagent information, step-by-step instructions for use, and valuable tips and hints. Print copies are available free of charge upon request and can also be downloaded at **www.stemcell.com/technical-resources/product-information.html**.

Free, On-Demand Training

Mouse CFU Assay

Learn to Set Up and Perform the CFU Assay for Mouse HSPCs

www.stemcell.com/hsc-mouse-training

Human CFU Assay

Get Step-by-Step Instruction on Set Up, Colony Identification, and Scoring

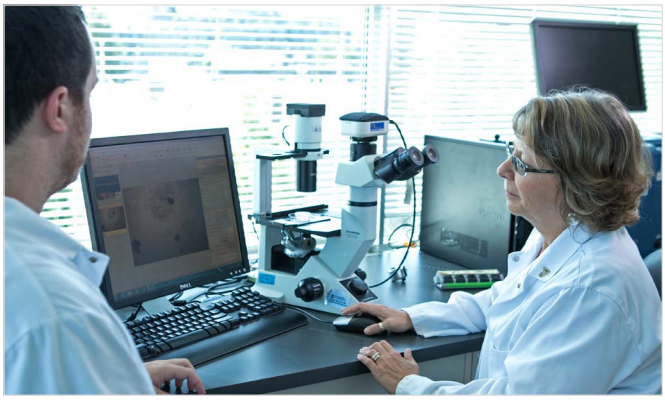
www.stemcell.com/hsc-training

CFU Assay Automation

Learn to Use STEMvision™ to Standardize CFU Scoring

www.stemcell.com/stemvision-training

Contract Assay Services



Contract Assay Services (CAS) is a contract research organization (CRO) established within STEMCELL Technologies that performs assay services based on in vitro and in vivo primary stem cell assays. Primary cells are thought to be more representative of in vivo functionality than cell lines, and can increase the biological relevance of in vitro assays. CAS specializes in providing the CFU assay for a variety of applications (see below), in addition to their portolio of characterized assays and custom solutions for your individual needs. Since 2000, CAS has performed such studies for over 120 pharmaceutical, biotechnology, government, and academic life science organizations worldwide, and provides exceptional service through frequent communication, quality products, and unparalleled expertise.

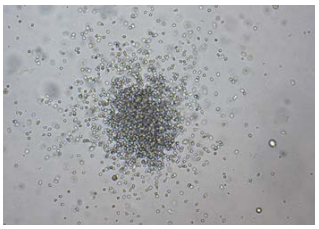
Stem and Progenitor Cell Assays

Our in vitro assays can help you determine a compound's inhibitory or stimulatory effects on hematopoietic progenitor cells:

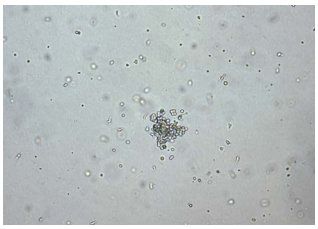
- CFU assays have been shown to be important in the evaluation of the potential inhibitory or stimulatory effects of a variety of compounds on hematopoietic and mesenchymal progenitor cells[72,78-86]
- CFU assays can be used to assess proliferation and differentiation of hematopoietic progenitor cells to determine IC_{50} and IC_{90} values
- CFU assays for myeloid progenitor cells have been validated for the determination of maximum tolerated dose by the European Centre for the Validation of Alternative Methods (ECVAM)
- Assays for CFU-GM and CFU-Mk have been shown to be predictive of clinical outcomes such as neutropenia and thrombocytopenia[72,81,82]

Our in vivo assays help you examine the effects of a compound on hematopoietic stem cells:

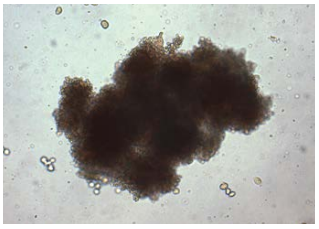
- Assess hematopoietic stem cell mobilization into the peripheral blood
- Determine kinetics of hematopoietic progenitor cell recovery following induction of myelosuppression



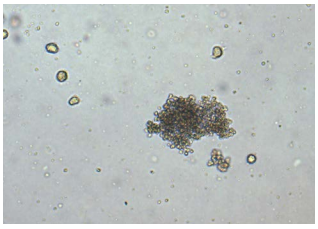
CFU-GM



CFU-GM with Inhibitory Compound



BFU-E



BFU-E with Inhibitory Compound

Figure 36. Effect of the Addition of an Inhibitory Compound on the Morphology of Human Bone Marrow CFU-GM and BFU-E Colonies

Interested in CAS?

Learn more about Contract Assay Services at STEMCELL Technologies

www.contractassay.com

Book a Consultation

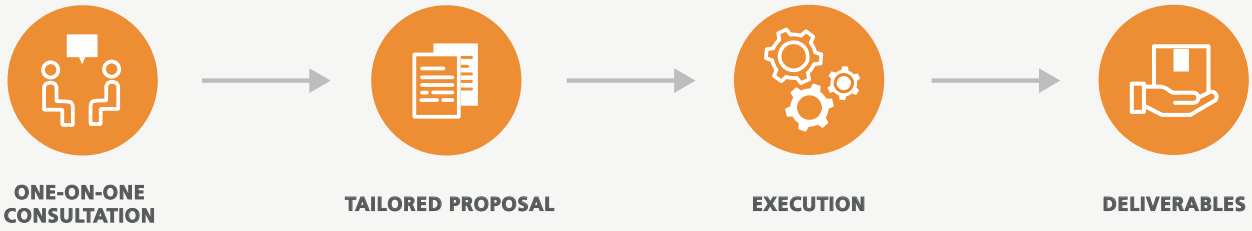
Contact us to find how we can help you meet your goals

www.stemcell.com/contact-cas

Services for Cell Therapy Program

If you are looking to use STEMCELL Technologies products as ancillary materials (AMs) or raw materials in the manufacture of hematopoietic cellular therapies, we can work with you to develop solutions for your specific needs, such as custom product development, higher compliance product manufacture, regulatory support, and customer documentation. Under the Services for Cell Therapy Program, STEMCELL may be able to support the use of our products as AMs (as defined under USP Chapter <1043> or Ph. Eur. General Chapter 5.2.12) under an approved Investigational New Drug (IND) application, Clinical Trial Authorization/Application (CTA), or equivalent regulatory filing. We have successfully supported over 100 clinical trials around the globe.

Overview of STEMCELL's Custom Services for Cell Therapies Process



Contract negotiations in parallel

Frequent communication with your dedicated program manager

Why Choose the Services for Cell Therapy Program?

GLOBAL EXPERIENCE. We have worked with customers around the world to meet their country- or region-specific regulatory requirements.

RELIABLE. Our stringent supplier qualification and management processes ensure consistent quality and a dependable supply of materials and services.

CUTTING-EDGE. We can support your workflows from start to finish with innovative, specialized reagents.

HIGH-QUALITY. In-house manufacturing and testing of select products under relevant cGMPs with the ability to support AM qualification under USP <1043> or Ph. Eur. 5.2.12.

ACCESSIBLE. Our network of qualified distribution centers enables worldwide delivery of our products.

Taking Your Research to the Clinic?

STEMCELL's Services for Cell Therapy program has a team of experts who can help support your regulatory filing by providing custom solutions such as quality documentation, additional product testing, and customized product manufacturing. To learn more about how we can support your preclinical and clinical research needs, visit us at **www.stemcell.com/services/cell-therapy.html**.

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