# An Integrated Workflow for Reprogramming Blood Cells

STEMCELL™ TECHNOLOGIES

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## An Integrated Workflow for the Generation of iPS Cells from Blood Cells

STEMCELL Technologies, Inc. offers a comprehensive line of products to support each step of your workflow, including the isolation and expansion of CD34$^+$ and erythroid progenitor cells, generation of induced pluripotent stem (iPS) cells, culturing and maintenance of embryonic stem (ES) and iPS cells, and differentiation to specialized cell types. Please visit us at **www.stemcell.com/hPSCworkflow** for additional information.

# Isolating and Reprogramming Blood-Derived Cells

## Reprogramming Peripheral Blood Cells

Human induced pluripotent stem (hiPS) cells are generated by reprogramming somatic cells to a pluripotent state, through the transient overexpression of key reprogramming factors.

Dermal fibroblasts were the first human cell type to be converted to iPS cells and are still one of the most common sources used for reprogramming experiments.[1,2] Since then, numerous primary cell sources such as keratinocytes, mesenchymal stem cells, T cells, B cells, hematopoietic progenitor cells, and urine epithelial cells have also been reprogrammed to hiPS cells.[1–8] The choice of starting cell type is influenced by factors such as availability of donor tissue from normal and diseased patients, invasiveness of sample collection procedures, genomic integrity, epigenetic memory, and reprogramming efficiency.

Peripheral whole blood (WB) is a popular tissue source for generating hiPS cells.[9] Blood collection is a well-established and minimally invasive procedure, and collected cells are naturally replaced as the tissue is self-renewing. Banked blood samples are also available for a wide variety of disease, age, gender and geographical subtypes. Since the cells are continually replenished from stem cells in the bone marrow, it is expected that they will contain fewer environment-associated point mutations than skin, which is exposed to long-term ultraviolet radiation.

However, WB contains a heterogeneous mixture of cell types, the most prevalent of which are enucleated (e.g. mature red blood cells (RBCs) and platelets) and therefore not suitable for reprogramming. The first step in preparing WB samples for reprogramming is therefore to separate the peripheral blood mononuclear cell (PBMC) fraction from the RBCs and platelets. This is generally done by density gradient centrifugation.

**Tip:** For a complimentary wallchart containing frequencies of cell types in human peripheral blood, visit: **www.stemcell.com/humancellfreq**.

The PBMC fraction consists of T and B cells, macrophages, monocytes, erythroid progenitors and rare circulating stem cells. Many of these cell types have been successfully reprogrammed with varying efficiencies.[9] While T and B cells are the most abundant cell type in the PBMC fraction and have been successfully reprogrammed, they contain V(D)J genomic rearrangements of the T-cell receptor or immunoglobulin loci, respectively. The ability to generate hiPS cells from specific T-cells has been utilized for proof of principle experiments in T-cell therapy,[10] but little is known about how these rearrangements may affect the function of other downstream cell lineages.

Less abundant cell types such as CD34$^+$ hematopoietic stem and progenitor cells and erythroid progenitor cells are attractive for reprogramming, due to their lack of genomic rearrangements and demonstrated reprogramming ability.[7,11] However, owing to their low frequency in whole blood, these cells need to be isolated from WB and/or expanded in vitro to obtain sufficient cell numbers for reprogramming.

### Integrated Sets of Tools for Reprogramming Blood Cells

**Erythroid Progenitor Reprogramming Kit**

- Enrich cells with **RosetteSep™** and **SepMate™**
- *No additional isolation step required*
- Expand erythroid cells with **StemSpan™ SFEM II** + **Erythroid Expansion Supplement**
- Reprogram cells with **ReproTeSR™**

**CD34$^+$ Progenitor Reprogramming Kit**

- Enrich cells with **RosetteSep™** and **SepMate™**
- Isolate CD34$^+$ cells with **EasySep™**
- Expand CD34$^+$ cells with **StemSpan™ SFEM II** + **CD34$^+$ Expansion Supplement**
- Reprogram cells with **ReproTeSR™**

*Note: EasySep™ magnet is not included. For more details on the reagents included in each kit, see page 13.*

## An Integrated Workflow

We have developed an integrated set of tools to facilitate the reprogramming of WB to hiPS cells. First, RBCs, platelets and lineage-committed cells are depleted from blood using a RosetteSep™ cocktail and SepMate™ density gradient centrifugation tubes. If further purification of progenitor cells is desired, CD34$^+$ cells can be isolated from the RosetteSep™-enriched fraction by positive selection using the immunomagnetic, column-free EasySep™ platform. Enriched cells can then be cultured in StemSpan™ medium supplemented with cytokine cocktails designed to promote the expansion of CD34$^+$ cells, or the expansion and subsequent lineage-specific differentiation of erythroid progenitor cells.

Once sufficient cell numbers have been generated, reprogramming can begin through transfection/transduction of reprogramming factors. Our xeno-free medium, ReproTeSR™, supports rapid and efficient feeder-free reprogramming of blood-derived cells. hiPS cells generated from this workflow can transition seamlessly to our TeSR™ family of hiPS cell maintenance media and the STEMdiff™ suite of products for directed differentiation.

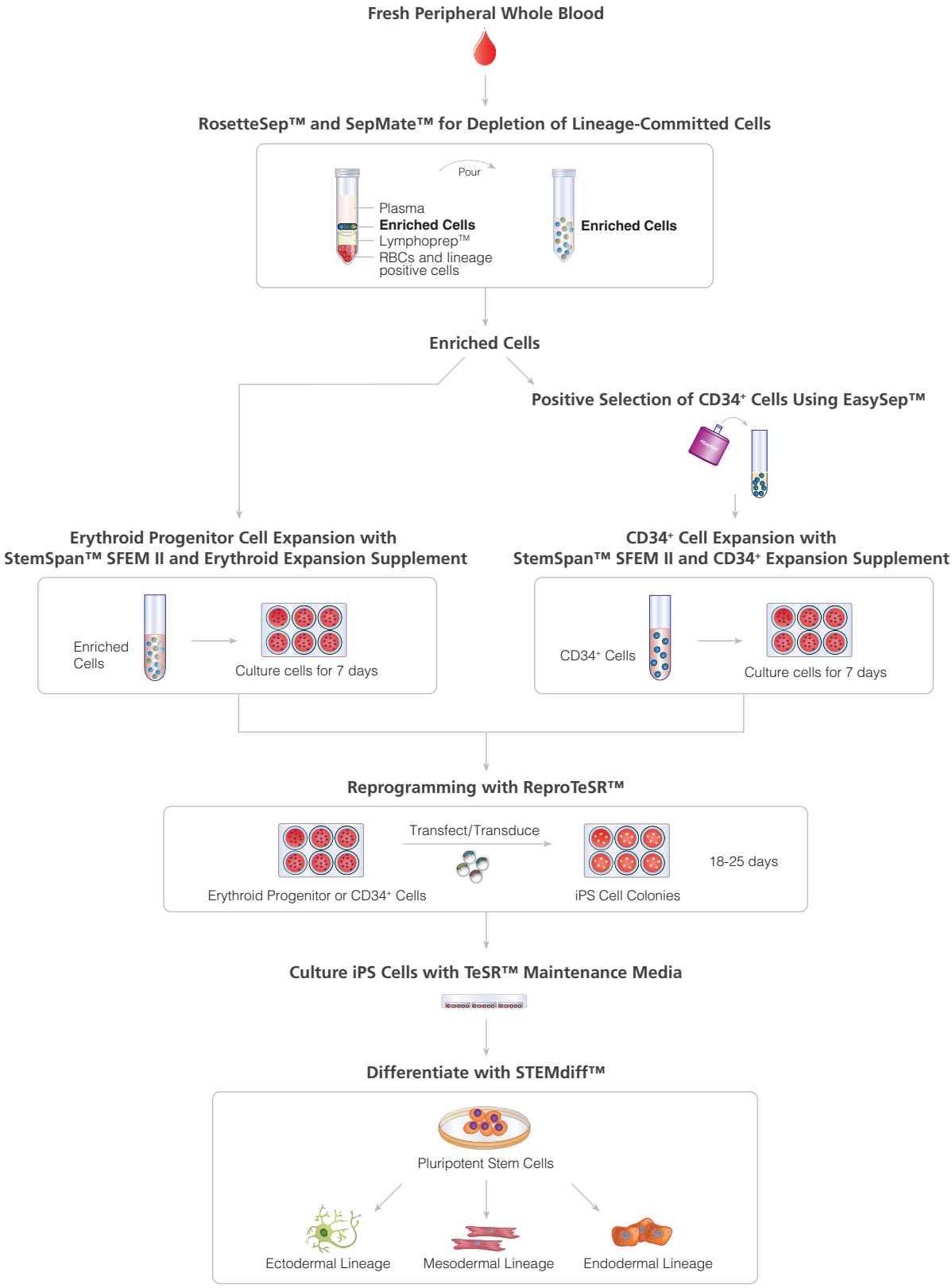


**Figure 1.** Workflow for Enrichment, Isolation, Expansion and Reprogramming of Erythroid Progenitor or CD34+ Cells

# Rapid Cell Enrichment

## Deplete Lineage-Committed Cells with RosetteSep™ and SepMate™

Successful generation of hiPS cells from WB can be achieved by first isolating PBMCs using density gradient centrifugation. This isolation step removes unwanted cells such as mature RBCs and platelets while retaining the desired progenitor cell population. PBMC isolation using density gradient centrifugation is traditionally a lengthy and technically difficult procedure. First, the blood has to be carefully layered on top of a density gradient medium. The sample is then centrifuged for at least 30 minutes (with no brake). Finally, PBMCs are delicately harvested from the density gradient medium and plasma interface. The isolation of PBMCs can take over 45 minutes and the challenging techniques of blood sample layering and PBMC interphase harvesting lead to significant variability between users.

**SepMate™** is a specialized tube with a unique insert that prevents layers from mixing so blood can be quickly pipetted or poured over the density gradient medium such as **Lymphoprep™**. Centifugation is cut down to just 10 minutes (with the brake on) and the resulting progenitor cells are simply poured into a new tube.

Mature cell types such as T- and B-cells isolated from WB may not be suitable sources for reprogramming, due to the presence of V(D)J rearrangements. These cell types can be quickly depleted during the density gradient centrifugation using **RosetteSep™** (Figures 2, 3). RosetteSep™ binds unwanted cells to RBCs, forming immunorosettes, which pellet during density gradient centrifugation. To remove T- and B-cells prior to erythroid cell expansion and differentiation, we recommend the **RosetteSep™ Human Progenitor Cell Basic Pre-Enrichment Cocktail**.

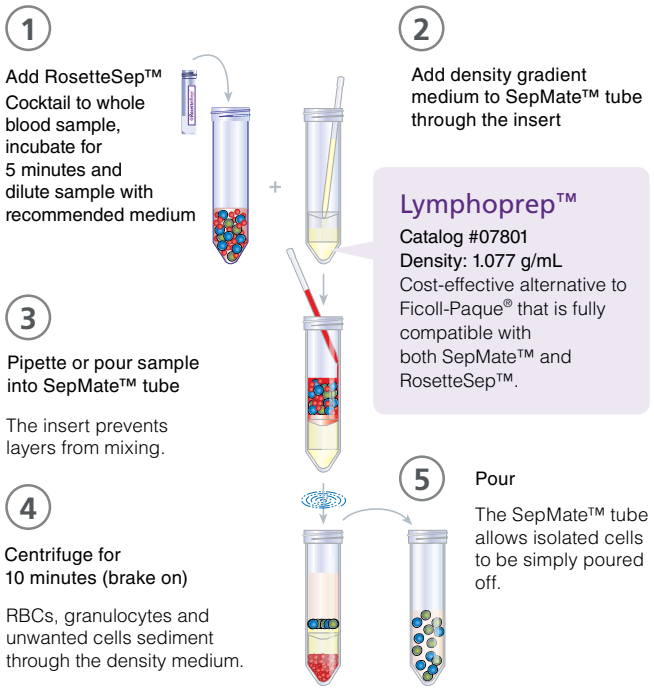


**Figure 2.** Schematic of Cell Enrichment with RosetteSep™ and SepMate™

*Note: Incubation and centrifugation times depicted in the schematic are typical for Rosettep™ Human Progenitor Cell Basic Pre-Enrichment Kit (Catalog #15226) used with SepMate™. Times may vary depending on the exact isolation protocol for each kit.*

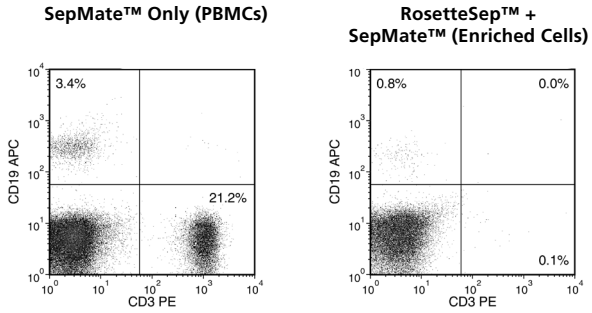


**Figure 3.** Depletion of T and B Cells from Whole Blood with RosetteSep™ Human Progenitor Cell Basic Pre-Enrichment Cocktail (Catalog #15226) and SepMate™ Tubes

PBMCs were enriched from whole blood using SepMate™ alone or lineage committed cells were depleted using SepMate™ and RosetteSep™ Enrichment Kit. In the example above the T cell ($CD3^+$) and B cell ($CD19^+$) population after depletion corresponds to <1%.

| PRODUCT | SIZE | CATALOG # |
|---|---|---|
| RosetteSep™ Human Progenitor Cell Basic Pre-Enrichment Kit | For labeling 1000 mL of blood | 15226 |
| SepMate™-15 (IVD)[1] | 100 tubes | 85415 |
| SepMate™-15 (RUO) | 100 tubes | 86415 |
| SepMate™-50 (IVD)[1] | 100 tubes | 85450 |
| SepMate™-50 (RUO) | 100 tubes | 86450 |
| Lymphoprep™ | 500 mL | 07851 |

1. SepMate™ (IVD) is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of MNCs from whole blood or bone marrow by density gradient centrifugation.

# Immunomagnetic CD34⁺ Cell Isolation

## Isolate Highly Purified CD34⁺ Cells With EasySep™

The **EasySep™ Complete Kit for Human Whole Blood CD34⁺ Cells** is a simple method for isolating highly purified CD34⁺ stem and progenitor cells (Figure 4,5). This kit contains both RosetteSep™ and EasySep™ components. First, the RosetteSep™ Human Hematopoietic Progenitor Enrichment Cocktail is used to deplete lineage-positive cells during density gradient centrifugation (as described on page 5). Next, the CD34⁺ cells are labeled with the EasySep™ Human CD34 Positive Selection cocktail and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while CD34⁺ cells remain in the tube.

| PRODUCT | SIZE | CATALOG # |
|---|---|---|
| EasySep™ Complete Kit for Human Whole Blood CD34⁺ Cells | For labeling 120 mL of whole blood | 15086 |


**Figure 4.** Schematic of a Typical CD34⁺ Cell Isolation with the EasySep™ Complete Kit for Human Whole Blood CD34⁺ Cells

*Note: Protocol depicted is for the EasySep™ purple magnet (Catalog #18000). Times may vary with other EasySep™ platforms.*

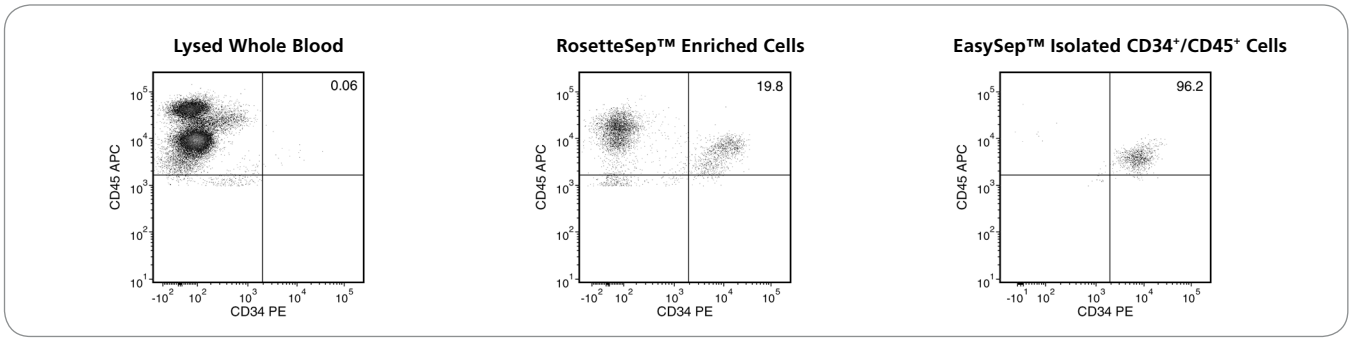


**Figure 5.** Typical Performance of the EasySep™ Complete Kit for Human Whole Blood CD34⁺ Cells (Catalog #15086)

Comparison of frequency of CD34⁺ CD45⁺ cells in lysed whole blood (<1%) and blood sample after performing first enrichment step with RosetteSep™ and after positive selection step with EasySep™. Starting with whole peripheral blood, the CD34⁺ cell content of the isolated fraction after the positive selection step is typically 95.1 ± 4.5% (gated on viable CD45⁺ cells; mean ± SD for the silver "The Big Easy" EasySep™ Magnet). In the above example, the purity of the final isolated fractions is 96.2%.

# Expansion of HSPCs in Culture

## Expand HSPCs with StemSpan™ SFEM II and Expansion Supplements

Hematopoietic stem and progenitor cells (HSPCs), including erythroid progenitor cells and CD34$^+$ cells, are excellent targets for reprogramming. However, due to the low frequency of these cells in PB (typically < 1% of PBMCs) and their low reprogramming efficiency, it is necessary to expand HSPCs in culture to obtain sufficient numbers of target cells for reprogramming. Erythroid progenitor cells or CD34$^+$ cells can be expanded by culturing blood-derived cells with **StemSpan™ Serum-Free Expansion Medium (SFEM) II** and specific **Supplements** (see below). StemSpan™ SFEM II, an enhanced version of StemSpan™ SFEM, is the preferred medium for CD34$^+$ and erythroid expansion cultures because it supports higher expansion than all leading hematopoietic media tested. StemSpan™ SFEM II contains pretested bovine serum albumin, human insulin and transferrin, and other supplements.

## Expansion of CD34$^+$ Cells

HSPCs can be expanded by culturing purified CD34$^+$ cells in **StemSpan™ SFEM II** supplemented with **StemSpan™ CD34$^+$ Expansion Supplement**. After culturing for 7 days, cell numbers can be expected to increase 20- to 50-fold with approximately 60% of the cells retaining expression of the CD34 antigen (Table 1).

| PRODUCT | SIZE | CATALOG # |
|---|---|---|
| StemSpan™ SFEM II | 100 mL | 09605 |
| | 500 mL | 09655 |
| StemSpan™ Erythroid Expansion Supplement (100x) | 1 mL | 02692 |
| StemSpan™ CD34$^+$ Expansion Supplement (10x) | 10 mL | 02691 |

Table 1. StemSpan™ SFEM II and CD34$^+$ Expansion Supplement Support Expansion of CD34$^+$ Cells

| | Pre-Expansion | Post-Expansion | | | |
|---|---|---|---|---|---|
| Sample | % CD34$^+$CD45$^+$ Cells | % CD34$^+$CD45$^+$ Cells | TNC Fold Increase | CD34$^+$CD45$^+$ Cells Fold Increase | # of CD34$^+$CD45$^+$ Cells Recovered / 50 mL Blood |
| 1 | 90 | 56 | 24 | 15 | $3.0 \times 10^5$ |
| 2 | 39 | 42 | 7 | 8 | $3.5 \times 10^4$ |
| 3 | 93 | 69 | 67 | 50 | $1.4 \times 10^6$ |
| 4 | 58 | 70 | 16 | 19 | $5.8 \times 10^5$ |
| 5 | 47 | 50 | 33 | 34 | $8.7 \times 10^5$ |
| 6 | 93 | 64 | 31 | 21 | $7.6 \times 10^5$ |
| 7 | 94 | 56 | 55 | 32 | $8.8 \times 10^5$ |
| 8 | 75 | 54 | 35 | 25 | $8.1 \times 10^5$ |
| 9 | 79 | 63 | 40 | 32 | $3.0 \times 10^5$ |
| 10 | 90 | 66 | 31 | 23 | $4.4 \times 10^5$ |
| 11 | 60 | 67 | 38 | 43 | $5.8 \times 10^5$ |
| 12 | 42 | 70 | 47 | 77 | $7.7 \times 10^5$ |
| **Average** | **72** | **60** | **35** | **32** | **$6.5 \times 10^5$** |
| 95% CL | 60 - 84 | 55 - 66 | 26 - 45 | 21 - 42 | 4.4 - 8.5 x $10^5$ |

TNC: Total Nucleated Cell

# Expansion of Erythroid Cells

Erythroid progenitor cells can be generated through the selective expansion and differentiation of HPSCs by culturing PBMCs in **StemSpan™ SFEM II** containing **StemSpan™ Erythroid Expansion Supplement.** Erythroid cells generated in these cultures are identified by expression of the transferrin receptor (CD71) and Glycophorin A (GlyA). $CD71^+GlyA^+$ cells make up the majority of expanded erythroid cells and consist mostly of (basophilic) erythroblasts. A smaller population of $CD71^+GlyA^{-/low}$ cells that contains pro-erythroblasts and more immature progenitor cells is also produced in these cultures (Figure 6).

Erythroid expansion cultures can be initiated with PBMCs (Table 2). However, mature T cells that are abundant in this cell fraction form a major contaminating cell type after culture. To avoid contamination of reprogramming cultures with T- and B-cells, erythroid cultures can be initiated with blood cells that have been depleted of these mature cells (with RosetteSep™) beforehand.

## Table 2. Generation of Erythroid Cells from PBMCs or Lineage-Depleted PB Cells in StemSpan™ SFEM II Containing StemSpan™ Erythroid Expansion Supplement

| | | Pre-Expansion | 7 Days Post-Expansion | | | | |
|---|---|---|---|---|---|---|---|
| **CELL SOURCE** | **n** | **% $CD71^+$ Cells** | **% $CD71^+$ Cells** | **% $CD3^+$ Cells** | **TNC Fold Increase** | **$CD71^+$ Cell Fold Increase** | **# $CD71^+$ Cells Recovered / 10ml of Blood** |
| Fresh PBMCs | 18 | **2.2** (0.01 - 8.9) | **68** (13 - 98) | **26** (3 - 81) | **0.9** (0.1 - 2.4) | **646** (2 - 10035) | **$3.6 \times 10^6$** ($7.0 - 110 \times 10^5$) |
| Frozen PBMCs | 3 | **2.1** (0.03 - 4.2) | **59** (28 - 83) | **38** (17 - 63) | **0.4** (0.3 - 0.7) | **310** (4 - 916) | **$3.4 \times 10^5$** ($6.3 - 46 \times 10^6$) |
| RosetteSep™ Lineage-Depleted Cells | 4 | **2.1** (0.5 - 4.8) | **90** (73 - 97) | **0.02** (0.01 - 0.03) | **0.7** (0.5 - 1.3) | **62** (7 - 116) | **$1.9 \times 10^6$** ($4.9 - 32 \times 10^5$) |

TNC: total nucleated cell
Average range shown in bold

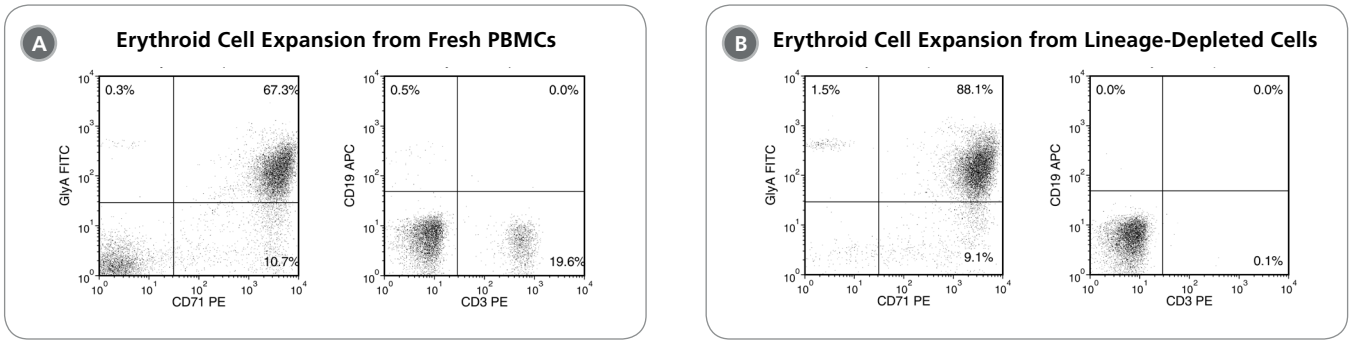


**Figure 6.** Erythroid Progenitor Cell Expansion and Differentiation in StemSpan™ SFEM II Containing Erythroid Expansion Supplement

**(A)** Isolated PBMCs were cultured for 7 days in SFEM II containing erythroid expansion supplement, and then examined by flow cytometry for erythroid progenitor cells, T-cells and B-cells. Representative plots illustrate that erythroid progenitor cells ($GlyA^+CD71^+$) increase after 7 days, though some T-cells ($CD3^+$) and B-cells ($CD19^+$) remain. **(B)** Use of the RosetteSep™ cocktail to deplete mature blood cells prior to expansion cultures leads to increased purity of erythroid progenitor cells and negligible contamination with lymphoid cells. *Note: same donor sample used for A and B.*

# Reprogram

## Reproducible Generation of iPS Cells from Blood-Derived Cells with ReproTeSR™

**ReproTeSR™** is a complete, defined, xeno-free and feeder-free reprogramming medium optimized for the generation of hiPS cells from erythroid or CD34⁺ cells expanded in vitro from PB.

ReproTeSR™ is intended for use during the induction phase of reprogramming (Figure 7) and yields more hiPS cell colonies than traditional KOSR-containing human embryonic stem (hES) cell medium. hiPS cell colonies generated with ReproTeSR™ express undifferentiated cell markers and exhibit more defined borders, compact morphology, and reduced differentiation.

ReproTeSR™ seamlessly integrates with RosetteSep™, SepMate™, EasySep™ and StemSpan™ products for isolation and expansion of hematopoietic cells, as well as TeSR™ and STEMdiff™ products for downstream maintenance and differentiation of hiPS cell lines.

### Advantages of ReproTeSR™

**DEFINED.** Feeder-free formulation facilitates reproducibly efficient human iPS cell generation.

**EASILY ESTABLISH iPS CELL LINES.** Rapid emergence of large colonies with high quality hiPS cell-like morphology facilitates identification and subcloning.

**INTEGRATED WORKFLOW.** Seamlessly integrates with STEMCELL products prior to reprogramming, and after hiPS cell generation for maintenance and differentiation.

| PRODUCT | SIZE | CATALOG # |
|---|---|---|
| ReproTeSR™ | 500 mL | 05926 |

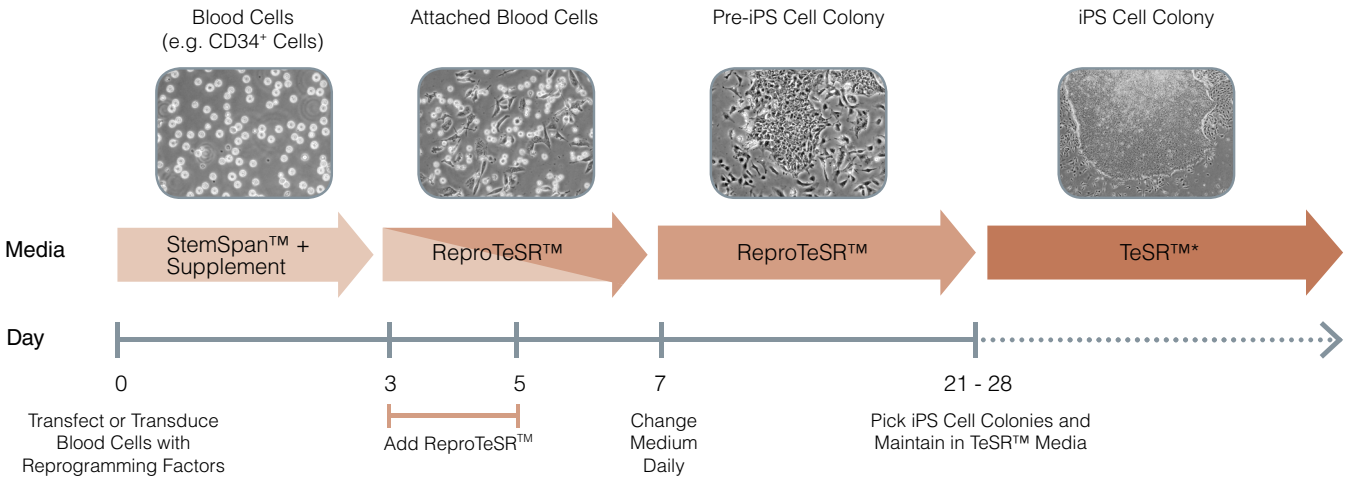


**Figure 7.** Schematic of ReproTeSR™ Reprogramming Timeline

ReproTeSR™ is used during the entire induction phase of reprogramming (days 3 to 21). On days 3 and 5, ReproTeSR™ is added to StemSpan™ growth media (in a fed-batch manner) to facilitate attachment of transfected cells. Attached cells are further cultured in ReproTeSR™ with daily full media changes until putative hiPS cell colonies emerge (days 21 to 28). hiPS cell colonies can then be isolated and propagated in TeSR™ medium (mTeSR™1, TeSR™2 or TeSR™-E8™).

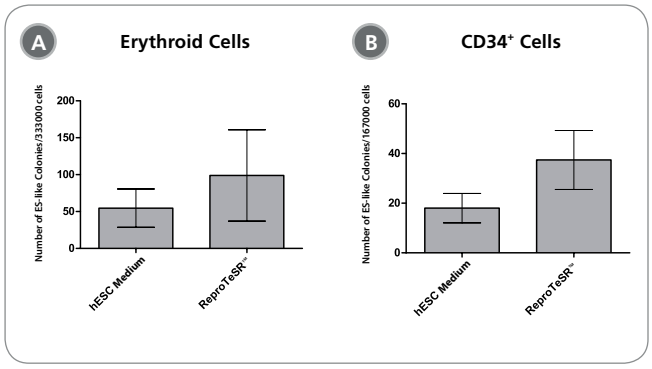


**Figure 8.** Blood Cell Reprogramming Efficiencies are Higher in ReproTeSR™ Medium Compared to in hES Cell Medium

Reprogramming efficiency of **(A)** erythroid cells or **(B)** CD34⁺ cells, using episomal reprogramming vectors is higher in ReproTeSR™ medium compared to in KOSR-containing hES cell medium. Data shown are mean ± SEM; erythroid cells n = 4; CD34⁺ cells n = 5.

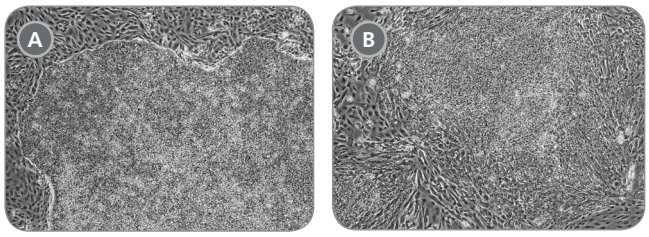


**Figure 9.** ReproTeSR™ Generates hiPS Cell Colonies with Superior Colony Morphology

Representative images of hiPS cell colonies generated from isolated CD34⁺ progenitor cells using **(A)** ReproTeSR™ and **(B)** hES cell medium. hiPS cell colonies produced using ReproTeSR™ exhibit more defined borders, compact morphology and reduced differentiation compared with hES cell medium. 200X magnification.

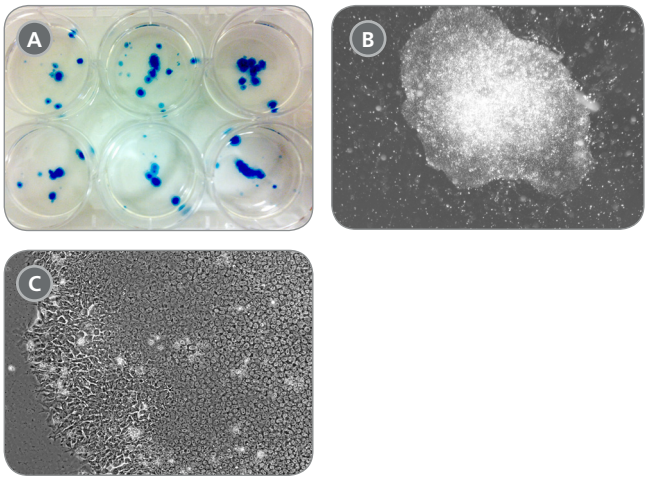


**Figure 10.** Generation of hiPS Cells from 1 mL of Peripheral Blood

Starting from 1mL of PB, PBMCs were enriched, and erythroid progenitors were expanded and reprogrammed in ReproTeSR™. **(A)** Approximately 75 hiPS cell-like colonies that were positive for alkaline phosphatase expression (blue) were generated. **(B,C)** hiPS cell colonies exhibit compact ES cell-like morphology with defined borders and high nuclear-to-cytoplasmic ratio. Representative images of generated hiPS cell colonies taken at 20X (B) and 400X (C) magnification are shown.

## Reprogramming Efficiency of CD34⁺ and Erythroid Progenitor Cells with ReproTeSR™

| CELL SOURCE | n | % Purity Post-Expansion | # of iPS Cell Colonies per 333,000 Cells | % Reprogramming Efficiency | # iPS Cell Colonies per mL Blood |
|---|---|---|---|---|---|
| CD34⁺ Cells | 5 | **62** (54 - 69) | **75** (30 - 163) | **0.02** (0.009 - 0.049) | **7.5** (1 - 11) |
| CD71⁺ Erythroid Cells (from Fresh PBMCs) | 7 | **73** (34 - 98) | **65** (6 - 282) | **0.02** (0.002 - 0.09) | **128** (6 - 599) |
| CD71⁺ Erythroid Cells (from RosetteSep™ Lineage-Depleted Cells) | 2 | **94** (92 - 96) | **47** (12 - 81) | **0.01** (0.004 - 0.024) | **35** (12 - 59) |

Average range shown in bold.

# Maintenance

## Maximize Your Pluripotential with TeSR™ Media for hPSC Culture

Maintenance of high quality human pluripotent stem cells (hPSCs) is critical to success in all applications of hPSC research. The TeSR™ family of feeder-free maintenance media can help you minimize variation in your research. Each TeSR™ medium is based on published formulations from the laboratory of Dr. James Thomson[12-14] and offers unique features to fit your research needs.

**mTeSR™1** is the most widely published feeder-free medium for hPSCs and is supported by protocols for a variety of applications including bioreactor expansion and single-cell cloning.[15-16]

**mTeSR™ Plus** is based on the mTeSR™1 formulation, with stabilized components, including FGF2, and enhanced pH buffering.

**TeSR™2** is a modified xeno-free formulation that replaces the bovine proteins in mTeSR™1 with recombinant human proteins. **TeSR™-E8™** is a simplified medium containing only the 8 most essential components required for maintenance of hPSCs. hiPS cell lines derived in ReproTeSR™ can be easily cultured in any TeSR™ media and maintain expression of undifferentiated cell markers.

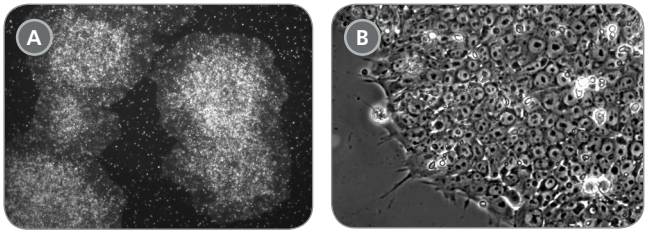


**Figure 11.** ReproTeSR™ Generates hiPS Cell Colonies with Superior Colony Morphology

**(A,B)** Representative images of hiPS cell colonies cultured in mTeSR™1 and generated from isolated CD34$^+$ progenitor cells using ReproTeSR™. hiPS cell colonies produced using ReproTeSR™ exhibit more defined borders, compact morphology and reduced differentiation compared with hES cell medium.

| PRODUCT | SIZE | CATALOG # |
|---|---|---|
| mTeSR™1 | 500 mL | 05850 |
| mTeSR™ Plus | 500 mL | 05825 |
| TeSR™2 | 500 mL | 05860 |
| TeSR™-E8™ | 500 mL | 05940 |
| ReLeSR™ | 100 mL | 05872 |
| | 500 mL | 05873 |

## mTeSR™1 is the Most Widely Published Feeder-Free hPSC Culture Medium

For more information and interviews with leading pluripotent stem cell researchers that use mTeSR™1, visit **www.stemcell.com/mTeSR1publications**.

## hPSC Passaging Without Manual Selection and Scraping

**ReLeSR™** is an animal component-free and enzyme-free reagent for dissociation and passaging of hPSCs as aggregates without manual selection or scraping. Passaging hPSCs with ReLeSR™ easily generates optimally-sized aggregates, while eliminating the hassle and variability associated with manual manipulation. By selectively detaching hPSCs, putative iPS cell colonies can be isolated from the surrounding differentiated cells.

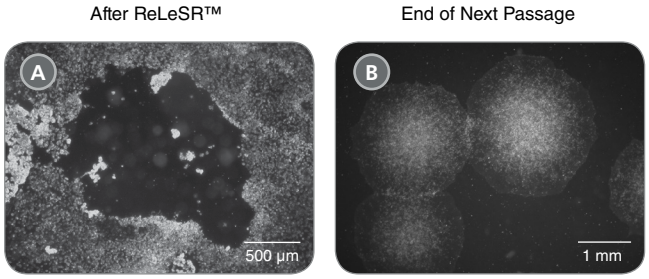


**Figure 12.** Easily Isolate Newly Generated Human iPS Cell Colonies with ReLeSR™ by Selectively Detaching Undifferentiated Cells and Leaving Non-Reprogrammed Cells Behind

(**A**) A TeSR™-E7™ reprogramming culture which has been treated with ReLeSR™ to detach the putative iPS cell colony, leaving the non-reprogrammed and differentiated cells behind. (**B**) Cultures contain a high proportion of undifferentiated cells by the end of the first passage.

# Differentiation

## Reduce Variability by Differentiating with STEMdiff™

Consistent differentiation of hPSC lines can be challenging. Reproducibly differentiate hPSCs to your downstream lineage of choice with the **STEMdiff™** suite of products. All STEMdiff™ products are defined, serum-free*, and include detailed, user-friendly protocols to help standardize your differentiation procedures.

### Ectodermal Lineage

The **STEMdiff™ Neural System** comprises multiple products for generating, expanding, differentiating, characterizing and cryopreserving neural progenitor cells. For a flexible approach to differentiation, **BrainPhys™ Neuronal Medium** is available, designed to better support in vitro neuronal function.

### Endodermal Lineage

The **STEMdiff™ Definitive Endoderm Kit** enables differentiation to multipotent definitive endoderm and is optimized for hPSCs cultured in mTeSR™1 or TeSR™-E8™.

The **STEMdiff™ Pancreatic Progenitor Kit** supports efficient generation of pancreatic progenitor cells from hPSCs.

### Mesodermal Lineage

Use **STEMdiff™ Mesoderm Induction Medium** for xeno-free differentiation to early mesoderm.

The **STEMdiff™ Hematopoietic Kit** is designed for the generation of hematopoietic progenitor cells from hPSCs.

The **STEMdiff™ Mesenchymal Progenitor Kit** is optimized for reproducible derivation of mesenchymal progenitor cells from hPSCs.

### Trilineage Differentiation

The **STEMdiff™ Trilineage Differentiation Kit** provides a simple cell culture assay to functionally and reproducibly validate the ability of new or established hPSCs to differentiate to the three germ layers.

### Flexible User-Directed Differentiation

**STEMdiff™ APEL™2** and **APEL™2-LI** media are lineage-neutral and allow researchers to customize their protocols by adding specific cytokines or small molecules to induce directed differentiation.

| LINEAGE | PRODUCT | SIZE | CATALOG # |
|---|---|---|---|
| Ectoderm | STEMdiff™ Neural Induction Medium | 250 mL | 05835 |
| | STEMdiff™ Neural Rosette Selection Reagent | 100 mL | 05832 |
| | STEMdiff™ Neural Progenitor Medium | 500 mL Kit | 05833 |
| | STEMdiff™ Neuron Differentiation Kit | 100 mL Kit | 08500 |
| | STEMdiff™ Neuron Maturation Kit | 100 mL Kit | 08510 |
| | STEMdiff™ Dopaminergic Neuron Differentiation Kit | 100 mL Kit | 08520 |
| | STEMdiff™ Dopaminergic Neuron Maturation Kit | 100 mL Kit | 08530 |
| | STEMdiff™ Astrocyte Differentiation Kit | 100 mL Kit | 08540 |
| | STEMdiff™ Astrocyte Maturation Kit | 100 mL Kit | 08550 |
| | BrainPhys™ Neuronal Medium | 500 mL | 05790 |
| | STEMdiff™ Human Neural Progenitor Antibody Panel | 1 Kit | 69001 |
| | STEMdiff™ Neural Progenitor Freezing Medium | 100 mL | 05838 |
| Endoderm | STEMdiff™ Definitive Endoderm Kit | 100 mL Kit | 05110 |
| | STEMdiff™ Definitive Endoderm Kit (TeSR™-E8™-Optimized) | 100 mL Kit | 05115 |
| | STEMdiff™ Pancreatic Progenitor Kit | 1 Kit | 05120 |
| Mesoderm | STEMdiff™ Mesoderm Induction Medium | 100 mL | 05220 |
| | STEMdiff™ Hematopoietic Kit | 1 Kit | 05310 |
| | STEMdiff™ Mesenchymal Progenitor Kit | 1 Kit | 05240 |
| Trilineage | STEMdiff™ Trilineage Differentiation Kit | 1 Kit | 05230 |
| User-Directed | STEMdiff™ APEL™2 | 100 mL | 05270 |
| | STEMdiff™ APEL™2-LI | 100 mL | 05271 |

*STEMdiff™ Astrocyte Differentiation Kit contains serum

# Product Information

**PRODUCT:** Erythroid Progenitor Reprogramming Kit
**CATALOG #:** 05924
**QUANTITY:** For reprogramming 10 mL of blood

Kit is supplied with:

| PRODUCT | SIZE |
|---|---|
| RosetteSep™ Human Progenitor Cell Basic Pre-Enrichment Cocktail | For labeling 100 mL of blood |
| SepMate™-15 | 4 tubes |
| Lymphoprep™ | 250 mL |
| StemSpan™ SFEM II | 100 mL |
| StemSpan™ Erythroid Expansion Supplement (100x) | 1 mL |
| ReproTeSR™ | 500 mL |

**PRODUCT:** CD34+ Progenitor Reprogramming Kit
**CATALOG #:** 05925
**QUANTITY:** For reprogramming 80 mL of blood

Kit is supplied with:

| PRODUCT | SIZE |
|---|---|
| EasySep™ Complete Kit for Human Whole Blood CD34+ Cells | For labeling 120 mL of blood |
| SepMate™-50 | 6 tubes |
| Lymphoprep™ | 250 mL |
| StemSpan™ SFEM II | 100 mL |
| StemSpan™ CD34+ Expansion Supplement (10x) | 10 mL |
| ReproTeSR™ | 500 mL |

## Additional Equipment


**EasySep™ Magnet**
CATALOG #18000

The EasySep™ Magnet is designed to hold one 5 mL polystyrene tube to isolate up to 2.5 x $10^8$ cells (or up to 5 x $10^8$ cells when isolating rare cells [e.g. CD34+]) per separation.

| PRODUCT NAME | CATALOG # |
|---|---|
| EasySep™ Magnet | 18000 |
| "The Big Easy" EasySep™ Magnet | 18001 |
| Easy 50 EasySep™ Magnet | 18002 |
| EasyEights™ EasySep™ Magnet | 18103 |
| EasyPlate™ EasySep™ Magnet | 18102 |

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# Accessory Products

## Primary Cells

Starting with the right primary cells as the foundation for your experiments is the best way to ensure success in the lab. STEMCELL Technologies offers a range of fresh and cryopreserved human primary cells* sourced from bone marrow, cord blood and peripheral blood that are ready for immediate use in reprogramming applications.

### Cryopreserved Human Primary Cell Products*

| PRODUCT | QUANTITY | CATALOG # |
|---|---|---|
| Human Peripheral Blood $CD34^+$ Cells | 1 million cells | 70040.2 |
| Human Peripheral Blood Mononuclear Cells | 100 million cells | 70025 |

### Fresh Human Primary Cell Products*

| PRODUCT | ANTICOAGULANT | QUANTITY | CATALOG # |
|---|---|---|---|
| Whole Peripheral Blood | CP2D | ≥ 450 mL | 70501 |
| | ACDA | ≥ 450 mL | 70504 |

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

## Matrices and Substrates

Defined and xeno-free **Vitronectin XF™** and **CellAdhere™ Laminin-521** support the growth and differentiation of hPSCs. They are effective alternatives to Matrigel® and, when used with TeSR™2 or TeSR™-E8™, provide completely defined and xeno-free hPSC culture systems under feeder-free conditions. These systems allow complete control over the culture environment, resulting in more consistent cell populations and reproducible results in downstream applications.

| PRODUCT | SIZE | CATALOG # |
|---|---|---|
| Vitronectin XF™ Kit* | 1 Kit | 07190 |
| Vitronectin XF™ | 2 mL | 07180 |
| CellAdhere™ Laminin-521 | 100 µg | 77003 |

*Kit contains Vitronectin XF™, CellAdhere™ Dilution Buffer, Gentle Cell Dissociation Reagent and Non-Tissue Culture-Treated 6-Well Plates.

## Cytokines

Cytokines are a commonly used tool in hPSC reprogramming protocols, ensuring consistency across multiple assays. For a complete listing of cytokines available for hPSC research, please visit **www.stemcell.com/cytokines**.

| PRODUCT | SIZE | CATALOG # |
|---|---|---|
| Human Recombinant bFGF | 50 µg | 78003 |
| Human Recombinant EGF | 500 µg | 78006 |

## Small Molecules

Small molecules are increasingly being used as tools to understand and regulate stem cell biology. Whether to affect reprogramming, self-renewal, or differentiation, the right small molecule can transform a research project. STEMCELL Technologies offers small molecules in high impact research to target the key pathways in stem cell biology.

| PRODUCT | PATHWAY/TARGET | CATALOG # |
|---|---|---|
| BIO | WNT pathway activator; Inhibits GSK3 | 72032 |
| CHIR99021 | WNT pathway activator; Inhibits GSK3 | 72052 |
| Dexamethasone | Glucocorticoid pathway activator; Activates glucocorticoid receptor | 72092 |
| Forskolin | cAMP pathway activator; Activates adenylyl cyclase | 72112 |
| PD0325901 | MEK/ERK pathway inhibitor; Inhibits MEK | 72182 |
| PD173074 | Tyrosine kinase inhibitor; Inhibits FGFR | 72162 |
| SB431542 | Activin/BMP/TGFβ pathway inhibitor; Inhibits ALK4, ALK5, and ALK7 | 72232 |
| Valproic Acid | Epigenetic modifier; Inhibits histone deacetylase (HDAC)1 | 72292 |

## Cryopreservation Media

Storage and cryopreservation of cells and tissues are important parts of the workflow for hPSC biological research.

**FreSR™** cryopreservation media are defined, serum-free and optimized for use with cells cultured with **TeSR™** maintenance media. Cells stored in **FreSR™** media have higher thawing efficiencies than cells frozen via conventional methods using serum. **mFreSR™** serum-free medium is optimized for cryopreservation of hPSCs as aggregates. **FreSR™-S** animal component-free medium is optimized for cryopreservation of cells in single-cell suspension.

**CryoStor®** is animal component-free, cGMP-manufactured with USP grade components, and designed to maintain high viability and maximize cell recovery after long-term storage. CryoStor® contains 10%, 5% or 2% dimethyl sulfoxide (DMSO) and provides a safe and protective environment for cells and tissues during the freezing, storage and thawing processes.

| PRODUCT | SIZE | CATALOG # |
|---|---|---|
| mFreSR™ | 50 mL | 05855 |
| | 10 x 5 mL Tubes | 05854 |
| FreSR™-S | 50 mL | 05859 |
| CryoStor® CS10 | 100 mL | 07930 |
| CryoStor® CS5 | 100 mL | 07933 |
| CryoStor® CS2 | 100 mL | 07932 |

## Antibodies

STEMCELL Technologies' high-quality primary and secondary antibodies are verified to work with our pluripotent stem cell reagents in specific applications, ensuring that your downstream cell analysis, including phenotyping and purity assessments, works consistently. For a complete listing of antibodies and conjugations available, please visit **www.stemcell.com/antibodies**.

| TARGET ANTIGEN | CLONE | ISOTYPE | CATALOG # |
|---|---|---|---|
| OCT4 (OCT3) | 3A2A20 | Mouse IgG2b | 60093 |
| SSEA-1 | MC-480 | Mouse IgM | 60060 |
| SSEA-3 | MC-631 | Rat IgM | 60061 |
| SSEA-4 | MC-813-70 | Mouse IgG3 | 60062 |
| SSEA-5 | 8e11 | Mouse IgG1 | 60063 |
| TRA-1-60 | TRA-1-60R | Mouse IgM | 60064 |
| TRA-1-81 | TRA-1-81 | Mouse IgM | 60065 |
| TRA-2-49 | TRA-2-49/6E | Mouse IgG1 | 60066 |
| TRA-2-54 | TRA-2-54/2J | Mouse IgG1 | 60067 |

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