IMMUNE CELL CULTURE

Activate, Expand, Maintain, and Differentiate Immune Cells

STEMCELL™ TECHNOLOGIES

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Immune Cell Culture

Culture Cells with ImmunoCult™

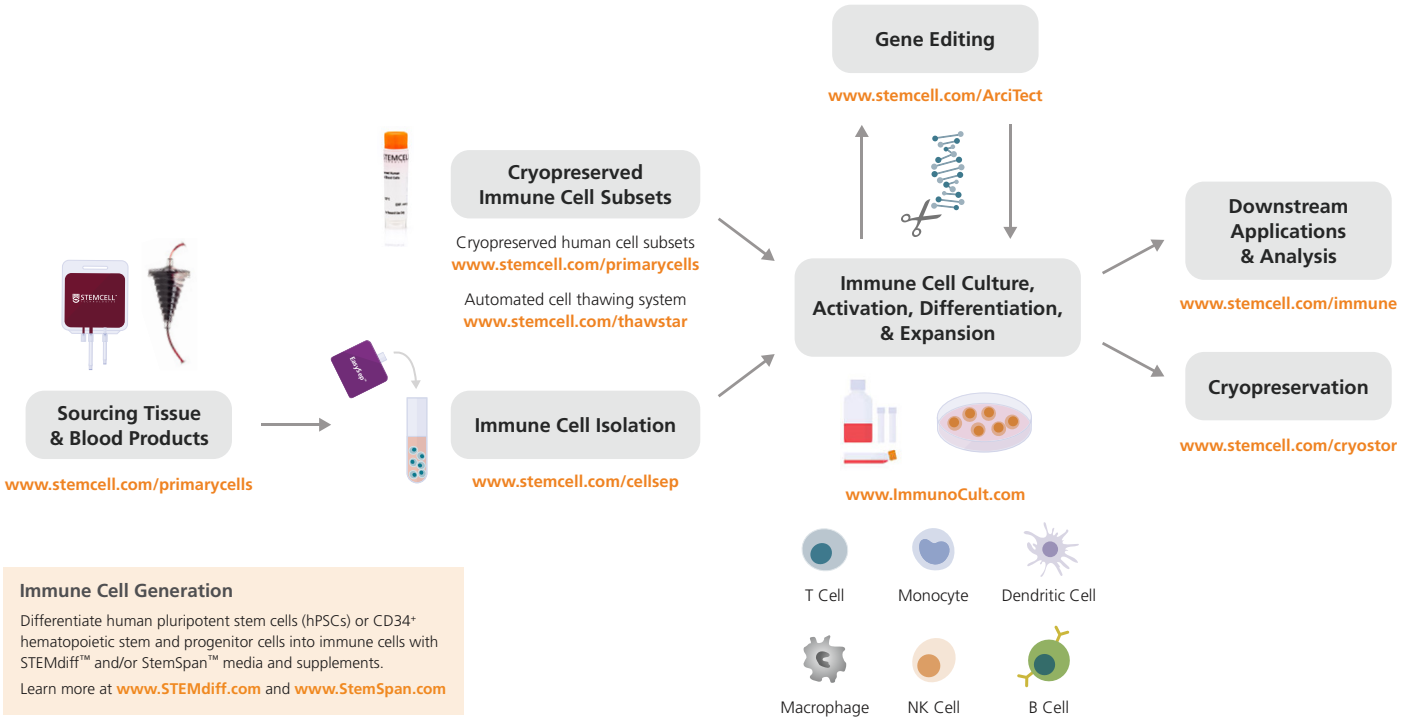
Activate, Expand, Maintain, and Differentiate Immune Cells

Ensure optimal activation, expansion, and differentiation of immune cell subsets by using STEMCELL Technologies' ImmunoCult™ cell culture media and supplements. ImmunoCult™ products allow you to culture various cell types, including monocytes, T cells, NK cells, B cells, dendritic cells, and macrophages, under defined stimulatory conditions for consistent and reliable results.

ImmunoCult™ products are part of a wider and complete immunology workflow of STEMCELL products.

Why Use ImmunoCult™?

- Activate, expand, or differentiate immune cells in culture conditions optimized to promote high yield and frequency
- Minimize variation by using serum-free culture conditions
- Consistently achieve high yields of immune cells with the desired phenotype and function
- Mix and match media, activators, and supplements to suit your specific research needs



Immune Cell Generation

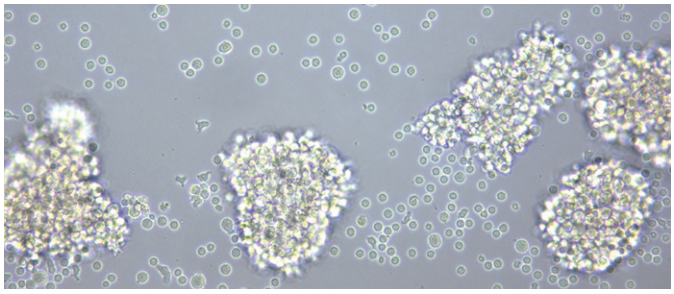
Differentiate human pluripotent stem cells (hPSCs) or CD34+ hematopoietic stem and progenitor cells into immune cells with STEMdiff™ and/or StemSpan™ media and supplements.

Learn more at www.STEMdiff.com and www.StemSpan.com

Figure 1. Products for Your Immune Cell Culture Workflow

Culture, Activate, Differentiate, and Expand T Cells

T cells play a vital role in regulating the immune response, and research on these cells is essential for understanding the complex responses to various pathogens and diseases, enabling development of new treatments and cures. Advance your basic T cell immunology and clinical T cell engineering research with products designed to ensure optimal culture conditions for human and mouse T cells. Culture cells under defined stimulatory conditions with specialized media, activators, and supplements to activate, edit, expand, or differentiate your cell population of interest.



ImmunoCult™ for Human T Cell Research

Culture and expand human T cells in vitro with serum-free and xeno-free medium. Use bead-free ImmunoCult™ T cell activators and differentiation supplements, which contain antibody complexes that target T cell receptors, to activate and expand T cells in culture. ImmunoCult™ T cell activators and differentiation supplements can be used in combination with ImmunoCult™-XF T Cell Expansion Medium or any other media for culturing human T cells.
The differentiation supplements contain a combination of human cytokines and a blocking antibody formulated to promote the robust activation, expansion, and differentiation of peripheral blood-derived human naïve $CD4^+$ T cells into Th1, Th2, or regulatory T cells.

ImmunoCult™ T cell media, activators, and supplements are compatible with many of our other upstream and downstream products, including cryopreserved T cells (Catalog #70024) and RosetteSep™ and EasySep™ cell separation reagents for isolating T cells from blood or leukapheresis products.

Why Use ImmunoCult™ for T Cell Research?

- Activate and expand T cells without the use of magnetic beads, feeder cells, or antigens
- Consistently expand T cells in serum- and xeno-free conditions
- Differentiate naïve T cells into Th1, Th2, or Treg cells

Why Use ImmunoCult™ T Cell Expansion Medium and Activators?

- Minimize variation with serum-free culture conditions
- Expand T cells at levels comparable to those in serum-containing media
- Obtain T cells that are able to produce cytokines, including IFN-gamma and IL-4
- Achieve higher yield of memory T cells in comparison with competitor differentiation kits
- Use cell culture reagents compatible with upstream cell isolation with EasySep™

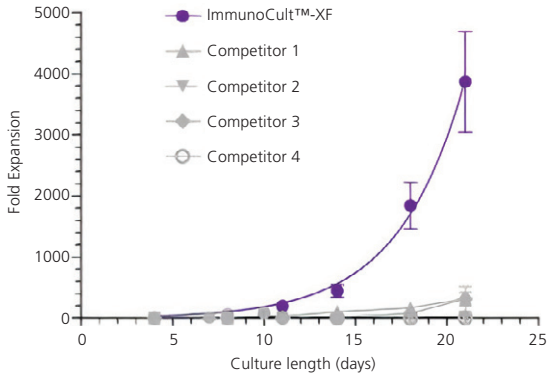


Figure 2. ImmunoCult™-XF T Cell Expansion Medium Supports Faster T Cell Expansion Than Other Serum-Free and Serum-Supplemented Media

T cells were isolated from human peripheral blood samples using the EasySep™ Human T Cell Isolation Kit (Catalog #17951), stimulated with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator (Catalog #10970), and cultured in ImmunoCult™-XF T Cell Expansion Medium supplemented with rhIL-2. T cells were stimulated with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator on Day 0 and every 7 to 8 days for the duration of the culture. T cells were analyzed on Days 4, 7, 8, 10, 11, 14, 18, and 21 for fold expansion relative to the initial cell seeding density. Compared to all competitor media tested, ImmunoCult™-XF T Cell Expansion Medium showed significantly higher expansion of total T cells. Competitors 1 to 4 include, in no particular order, X-VIVO™ 15 (Lonza), AIM V® Medium (Life Tech), CellGro® DC Medium (CellGenix), and RPMI 1640 + serum. Each data point represents the mean fold expansion ± SEM at the specified time points ($p < 0.05$ for ImmunoCult™-XF versus all media for Days 8, 11, 14, 18, and 21; tested using two-tailed, paired t-test with unequal variance; n = 6 to 19 donors). The average fold expansion of T cells in ImmunoCult™-XF T Cell Expansion Medium were 15-fold on Day 7, 80-fold on Day 10, 450-fold on Day 14, and 4,000-fold on Day 21.

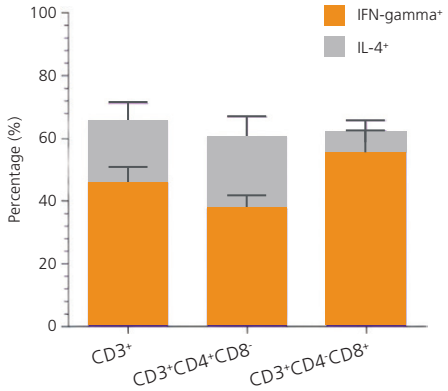


Figure 3. T Cells Expanded in ImmunoCult™-XF T Cell Expansion Medium and Activators Produce Intracellular IFN-gamma and IL-4

T cells were isolated from human peripheral blood samples using the EasySep™ Human T Cell Isolation Kit (Catalog #17951), stimulated with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator (Catalog #10970), and cultured in ImmunoCult™-XF T Cell Expansion Medium (Catalog #10981) supplemented with rhIL-2. T cells were stimulated with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator on Day 0 and every 7 to 8 days for the duration of the culture. On Day 21, T cells were harvested and analyzed for intracellular IFN-gamma and IL-4 after stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin for 4 hours, and with Brefeldin A for 2 hours. The production of IFN-gamma and IL-4 in $CD3^+$, $CD3^+CD4^+CD8^-$, and $CD3^+CD4^-CD8^+$ cells was determined. Each stacked column with error bars represents the mean ± SEM (n = 9 donors).

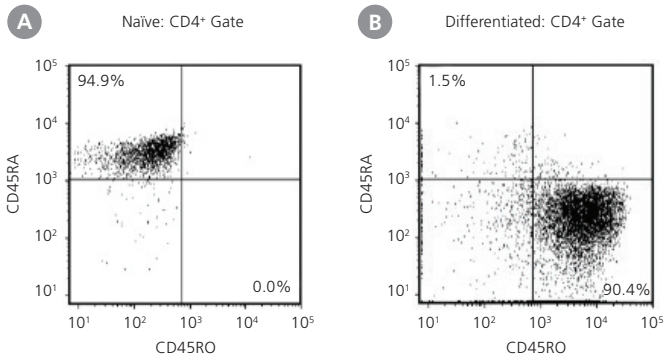


Figure 4. ImmunoCult™ Human Th1 Differentiation Supplement Produces Differentiated $CD4^+CD45RA^-CD45RO^+$ Cells Under Th1 Polarizing Conditions

Naïve $CD4^+$ T cells were isolated from human peripheral blood samples using the EasySep™ Human Naïve $CD4^+$ T Cell Isolation Kit (Catalog #19555). Cells were stimulated with ImmunoCult™ Human CD3/CD28 T Cell Activator (Catalog #10971) and ImmunoCult™ Human Th1 Differentiation Supplement (Catalog #10973), cultured in ImmunoCult™-XF T Cell Expansion Medium (Catalog #10981) for 7 days. Shown are flow cytometry results of cell samples stained and analyzed for expression of CD45RA and CD45RO (A) before and (B) after 7 days of culture. After 7 days, the majority of cells in culture display a memory T cell phenotype ($CD45RA^-CD45RO^+$).

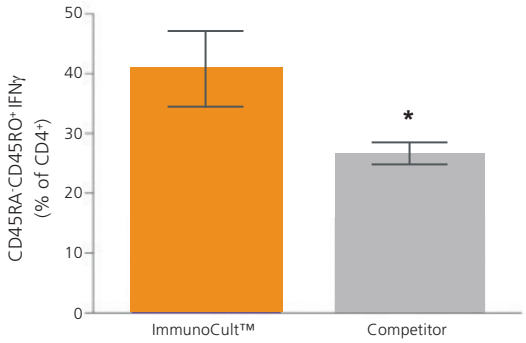


Figure 5. Culture with ImmunoCult™ Human Th1 Differentiation Supplement Produces More IFNγ-Expressing Cells Than Culture Under Competitor Conditions

Naïve $CD4^+$ T cells were cultured for 5 days in ImmunoCult™ activator, supplement, and medium, or competitor medium and activator using the protocol described in Figure 4. After culture, differentiated $CD4^+$ T cells ($CD45RA^-CD45RO^+$) were analyzed for IFNγ and IL-4 (not shown) expression, following stimulation with PMA and Ionomycin for 4 hours. A greater percentage of cells generated with ImmunoCult™ expressed intracellular IFNγ compared to those generated under competitor conditions, while no significant difference was found in the percentage of cells that expressed intracellular IL-4, with very low numbers of cells expressing IL-4 for both ImmunoCult™ and competitor. Bars represent the differences in the percentage of naïve and differentiated T cells in ImmunoCult™ (purple) and competitor (gray) culture conditions (mean ± SEM; *$p < 0.05$; paired t-test on logit transformed data; n = 4).

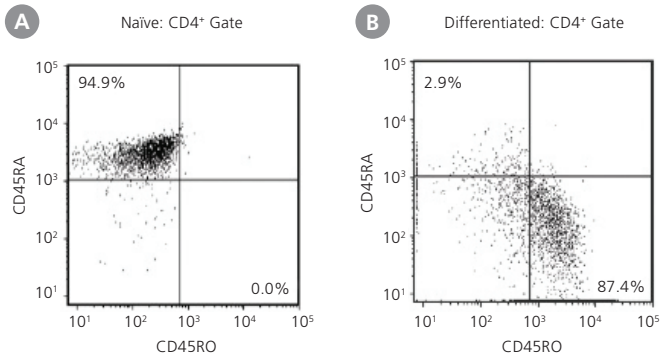


Figure 6. ImmunoCult™ Human Th2 Differentiation Supplement Produces Differentiated $CD4^+CD45RA^-CD45RO^+$ Cells Under Th2 Polarizing Conditions

Naïve $CD4^+$ T cells were isolated from human peripheral blood samples using the EasySep™ Human Naïve $CD4^+$ T Cell Isolation Kit (Catalog #19555). Cells were stimulated with ImmunoCult™ Human CD3/CD28 T Cell Activator (Catalog #10971) and ImmunoCult™ Human Th2 Differentiation Supplement (Catalog #10975), cultured in ImmunoCult™-XF T Cell Expansion Medium (Catalog #10981) for 14 days. Shown are flow cytometry results of cell samples stained and analyzed for expression of CD45RA and CD45RO (A) before and (B) after 14 days of culture. After 14 days the majority of cells in culture display a memory T cell phenotype ($CD45RA^-CD45RO^+$).

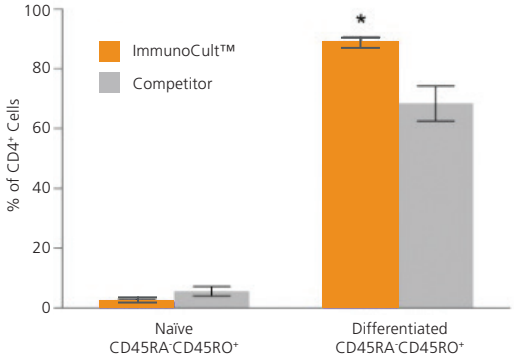


Figure 7. ImmunoCult™ Human Th2 Differentiation Supplement Produces More Differentiated $CD4^+CD45RA^-CD45RO^+$ Cells Under Th2 Polarizing Conditions Than Competitor

Naïve $CD4^+$ T cells were isolated from human peripheral blood samples using EasySep™ Human Naïve $CD4^+$ T Cell Isolation Kit (Catalog #19555). Cells were then cultured with ImmunoCult™ Human CD3/CD28 T Cell Activator (Catalog #10971) and ImmunoCult™ Human Th2 Differentiation Supplement (Catalog #10975) in ImmunoCult™ media. Differentiated cells ($CD45RA^-CD45RO^+$) were compared following staining for CD45RA and CD45RO, and analyzed by flow cytometry. The percentage of cells expressing a naïve phenotype was similar and the percentage of cells expressing a differentiated phenotype was significantly higher with ImmunoCult™ than with competitor medium and activator. Bars represent the differences in the percentage of naïve and mature T cells in ImmunoCult™ (purple) and competitor (gray) culture conditions (mean ± SEM; *$p < 0.05$; paired t-test on logit transformed data; n = 4).

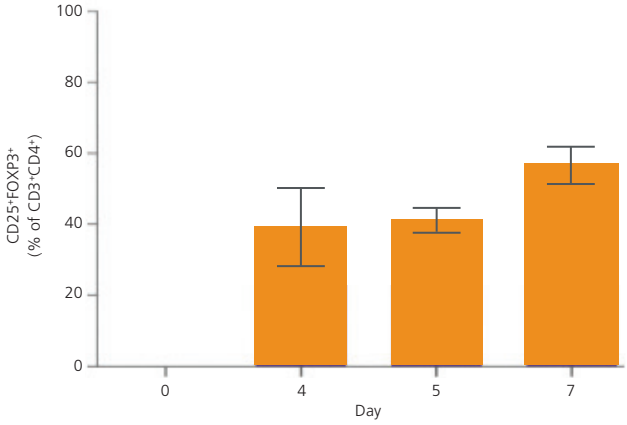


Figure 8. ImmunoCult™ Human Treg Differentiation Supplement Produces $FOXP3^+$ Cells Under Treg Polarizing Conditions

Naïve $CD4^+$ T cells were cultured for 7 days in ImmunoCult™ activator (Catalog #10970), supplement (Catalog #10977), and medium (Catalog #10981) using the protocol described in the product information sheet. Cells were stained for CD4, CD25, and FOXP3 and analyzed on Days 0, 4, 5, and 7 by flow cytometry. Each bar represents the mean percentage of $CD3^+CD4^+$ cells expressing both CD25 and FOXP3 ($CD3^+CD4^+CD25^+FOXP3^+$) (mean ± SEM; n = 4 - 20 donors).

TECHNICAL BULLETIN

Optimization of Human T Cell Expansion Protocol: Effects of Early Cell Dilution

www.stemcell.com/tcellexpansion

WALLCHART

Production of CAR T Cells

www.stemcell.com/cartcell-wallchart

ImmunoCult™ for Mouse T Cell Research

Differentiate mouse T cells with ImmunoCult™ supplements. These differentiation supplements contain recombinant mouse cytokines and blocking monoclonal antibodies formulated to promote the differentiation of spleen-derived mouse naïve CD4+ T cells into Th1, Th2, or regulatory T (Treg) cells. ImmunoCult™ differentiation supplements are intended for use with RPMI 1640 Medium containing fetal bovine serum and other additives, as well as anti-mouse CD3 and CD28 monoclonal antibodies as activating agents.

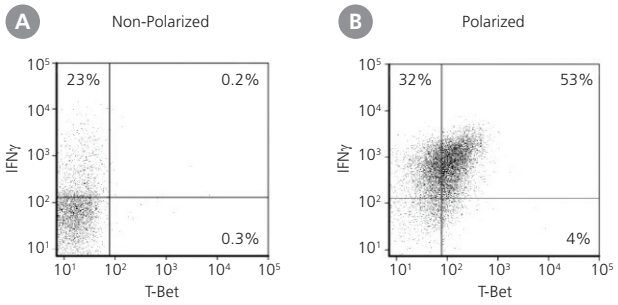


Figure 9. ImmunoCult™ Mouse Th1 Differentiation Supplement Produces CD4+IFNγ+T-Bet+ Cells Under Th1 Polarizing Conditions

Naïve CD4+ T cells were isolated from mouse splenocytes using the EasySep™ Mouse Naïve CD4+ T Cell Isolation Kit (Catalog #19765), activated with plate-bound anti-CD3 and soluble anti-CD28, and cultured in medium alone (non-polarized cultures) or medium supplemented with ImmunoCult™ Mouse Th1 Differentiation Supplement (polarized cultures) for 5 days. Cells were subsequently stimulated with PMA/Ionomycin, in conjunction with Brefeldin A treatment, and stained with anti-CD4, anti-IFNγ, anti-T-bet, and a viability dye, and analyzed by flow cytometry. Shown is the expression of IFNγ and T-bet, back-gated on viable CD4+ cells from (A) non-polarized or (B) polarized cultures. The mean proportion of CD4+IFNγ+T-Bet+ cells is significantly higher in cells cultured in ImmunoCult™ Mouse Th1 Differentiation Supplement (44 ± 6%) compared to non-polarized cells (2 ± 1%; $p < 0.001$; $n = 13$). Data from experimental groups were compared using a paired T-test.

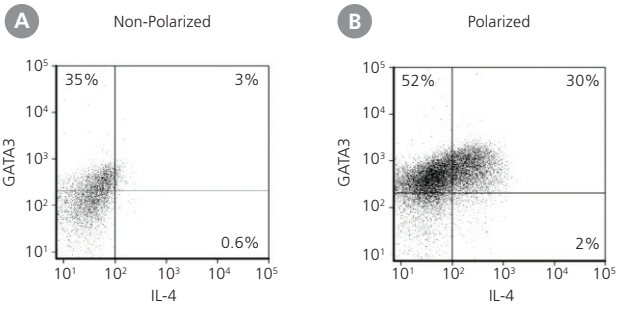


Figure 10. ImmunoCult™ Mouse Th2 Differentiation Supplement Produces CD4+GATA3+IL-4+ Cells Under Th2 Polarizing Conditions

Naïve CD4+ T cells were isolated from mouse splenocytes using the EasySep™ Mouse Naïve CD4+ T Cell Isolation Kit (Catalog #19765), activated with plate-bound anti-CD3 and soluble anti-CD28, and cultured in medium alone (non-polarized cultures) or in medium supplemented with ImmunoCult™ Mouse Th2 Differentiation Supplement (polarized cultures) for 6 days. Cells were subsequently stimulated with PMA/Ionomycin, in conjunction with monensin treatment, and stained with anti-CD4, anti-IL-4, anti-GATA3, and a viability dye, and analyzed by flow cytometry. Shown is the expression of GATA3 and IL-4 back-gated on viable CD4+ cells from (A) non-polarized or (B) polarized cultures. The mean proportion of CD4+IL-4+GATA3+ cells is significantly higher in cells cultured in ImmunoCult™ Mouse Th2 Differentiation Supplement (25 ± 3%) compared to non-polarized cells (4 ± 1%; $p < 0.001$; $n = 10$). Data from experimental groups were compared using a paired T-test.

Why Use ImmunoCult™ Differentiation Supplements?

- Induce Th1, Th2, or Treg cells from naïve CD4+ T cells isolated from mouse spleen
- Add 100x concentrated supplements directly to the medium
- Use cell culture reagents compatible with upstream cell isolation with EasySep™

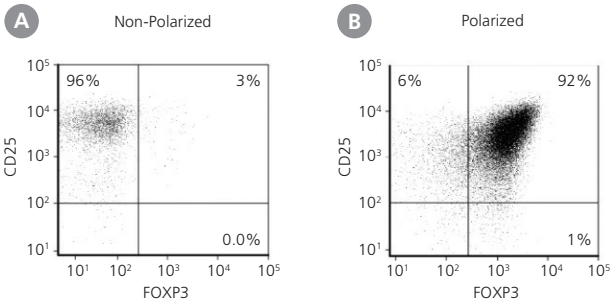


Figure 11. ImmunoCult™ Mouse Treg Differentiation Supplement Produces CD4+CD25+FOXP3+ Cells Under Treg Polarizing Conditions

Naïve CD4+ T cells were isolated from mouse splenocytes using the EasySep™ Mouse Naïve CD4+ T Cell Isolation Kit (Catalog #19765), activated with plate-bound anti-CD3 and soluble anti-CD28, and cultured in medium alone (non-polarized cultures) or in medium supplemented with ImmunoCult™ Mouse Treg Differentiation Supplement (polarized cultures) for 6 days. Cells were subsequently stained with anti-CD4, anti-CD25, anti-FOXP3, and a viability dye, and analyzed by flow cytometry. Shown is the expression of CD25 and FOXP3 back-gated on viable CD4+ cells from (A) non-polarized or (B) polarized cultures. The mean proportion of CD4+FOXP3+ cells is significantly higher in cells cultured in ImmunoCult™ Mouse Treg Differentiation Supplement (91 ± 2%) compared to non-polarized cells (2 ± 0.4%; $p < 0.001$; $n = 14$). Data from experimental groups were compared using a paired T-test.

WALLCHART

Frequencies & Percentages of Mouse Immune Cell Types

www.stemcell.com/mouse-cell-frequencies

ImmunoCult™ Human T Cell Products

Product	Catalog #	Size
ImmunoCult™-XF T Cell Expansion Medium	10981	500 mL
ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator	10970	2 mL
	10990	10 mL (5 x 2 mL)
ImmunoCult™ Human CD3/CD28 T Cell Activator	10971	2 mL
	10991	10 mL (5 x 2 mL)
ImmunoCult™ Human Th1 Differentiation Supplement	10973	1 mL
ImmunoCult™ Human Th2 Differentiation Supplement	10975	1 mL
ImmunoCult™ Human Treg Differentiation Supplement)	10977	1 mL

ImmunoCult™ Mouse T Cell Products

Product	Catalog #	Size
ImmunoCult™ Mouse Th1 Differentiation Supplement	10953	1 mL
ImmunoCult™ Mouse Th2 Differentiation Supplement	10955	1 mL
ImmunoCult™ Mouse Treg Differentiation Supplement	10957	1 mL

Culture and Expand B Cells

The ability to culture and expand B cells in vitro has become a useful tool to study human immunity, B cell biology, and vaccine and therapeutic antibody development. Culture and expand B cells in the absence of serum, feeder cells, or specialized culture plates with the human and mouse ImmunoCult™ B cell expansion kits. The components in these kits work together to ensure consistent activation and expansion of B cells and their maturation to plasma cells. Expanded B cells can be harvested and used directly in downstream applications. The ImmunoCult™ B cell expansion kits are compatible with many of our upstream and downstream tools. For example, you can use EasySep™ cell separation kits to isolate a range of human or mouse B cell subsets—such as pan-B cells, memory B cells, and naïve B cells—and then immediately expand the cells with ImmunoCult™ Human B Cell Expansion Kit or ImmunoCult™ Mouse B Cell Expansion Kit.

ImmunoCult™ media, activators, and supplements for B cells are compatible with many of our other upstream and downstream products, including cryopreserved B cells (Catalog #70023) and RosetteSep™ and EasySep™ cell separation reagents for isolating B cells from blood, leukapheresis products, or mouse tissue.

Why Use ImmunoCult™ to Expand Human and Mouse B Cells?

- Expand B cells in culture conditions optimized to promote yield and frequency
- Achieve robust in vitro expansion without serum, feeder cells, or specialized culture plates
- Obtain consistent yields of expanded human or mouse B cells
- Use cell culture reagents compatible with upstream cell isolation with EasySep™

ImmunoCult™ for Human B Cell Research

Reliably expand human B cells that are ready for use in downstream assays and applications—without the use of serum, specialized cultureware, or problematic feeder cells. Use ImmunoCult™ Human B Cell Expansion Kit to ensure consistent activation and expansion of human B cells and their maturation to plasma cells.

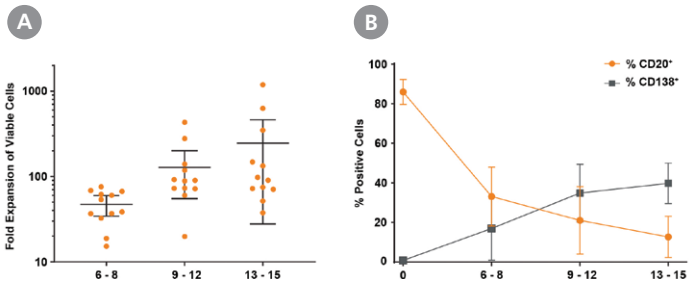


Figure 12. Expansion and Maturation of Human B Cells with ImmunoCult™ Human B Cell Expansion Kit

B cells isolated from human peripheral blood mononuclear cells (PBMS; leukopak) using EasySep™ Human Pan-B Cell Enrichment Kit (Catalog #19554) were seeded at 1 x 10^5 cells/well in 24-well tissue culture plates with ImmunoCult™-ACF Human B Cell Expansion Supplement and ImmunoCult™-XF B Cell Base Medium included in the ImmunoCult™ Human B Cell Expansion Kit. The cells were passaged every 3 - 4 days. (A) Fold expansion of viable cells is shown for n = 12 donors, with bars representing the mean and 95% confidence level (range 38- to 1190-fold at Day 14 ± 1 day). (B) Expression of CD138 and CD20 was analyzed by flow cytometry at each timepoint (data represent % positive viable cells; mean ± 1 SD). The observed changes indicate maturation of B cells to plasma cells/blasts.

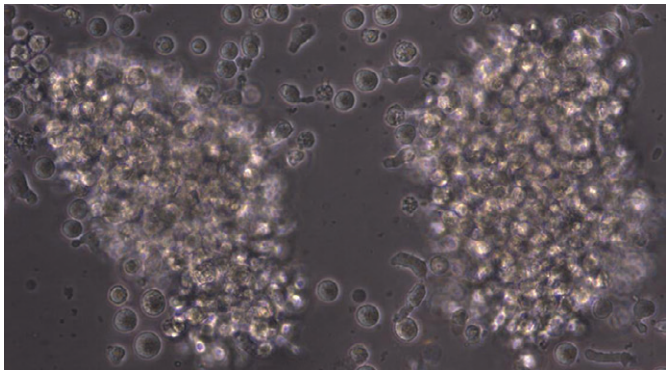


Figure 13. Light Microscopy Image of Cultured Human B Cells

B cells isolated from human PBMCs (leukopak) using EasySep™ Human Pan-B Cell Enrichment Kit were seeded at 1 x 10^5 cells/well in a 24-well tissue culture plate and cultured with the ImmunoCult™ Human B Cell Expansion Kit. The cells were passaged on Day 4 after seeding and imaged at 40X magnification on Day 6.

ImmunoCult™ for Mouse B Cell Research

Consistently expand mouse B cells in serum-free conditions without the use of problematic feeder cells. Use ImmunoCult™ Mouse B Cell Expansion Kit to create optimized culture conditions for expanding B cells from mouse splenocytes with high yields.

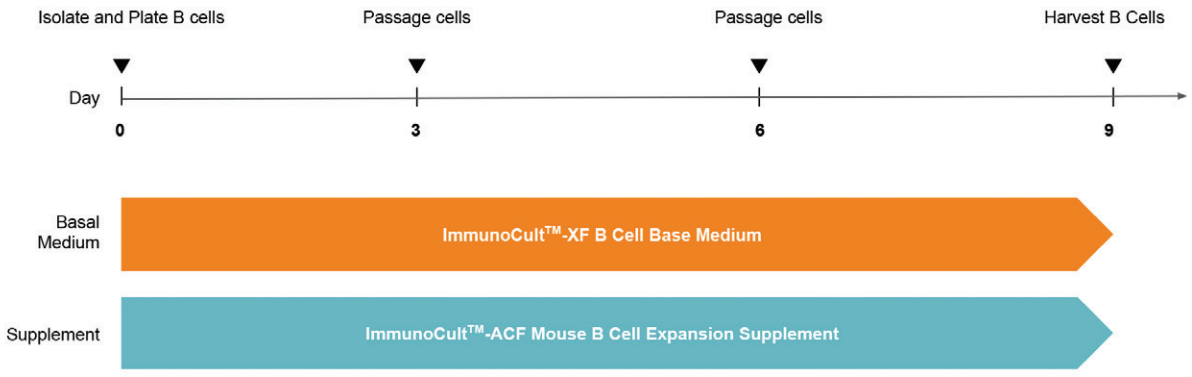


Figure 14. Protocol Diagram for Culturing Mouse B Cells with ImmunoCult™ Mouse B Cell Expansion Kit

B cells isolated from mouse spleen using EasySep™ Mouse Pan-B Cell Isolation Kit are cultured in complete Mouse B Cell Expansion Medium (ImmunoCult™-XF B Cell Base Medium + ImmunoCult™-ACF Mouse B Cell Expansion Supplement). B cells can be harvested on Day 9 for analysis or at earlier time points depending on the application.

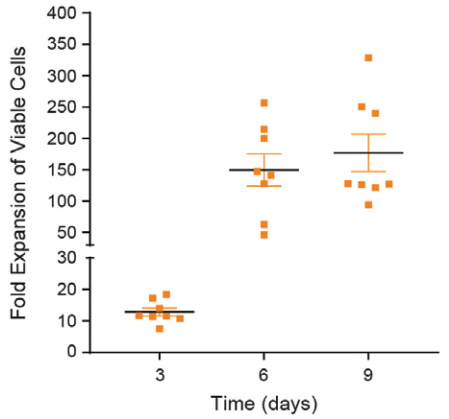


Figure 15. Expansion of Mouse B Cells with ImmunoCult™ Mouse B Cell Expansion Kit

B cells isolated from mouse spleen using EasySep™ Mouse Pan-B Cell Isolation Kit were cultured as described in Figure 14. Fold expansion of viable cells is shown with bar graphs representing the mean ± SEM (n = 8). B cells expanded 176.9 ± 29.8-fold after 9 days of culture.

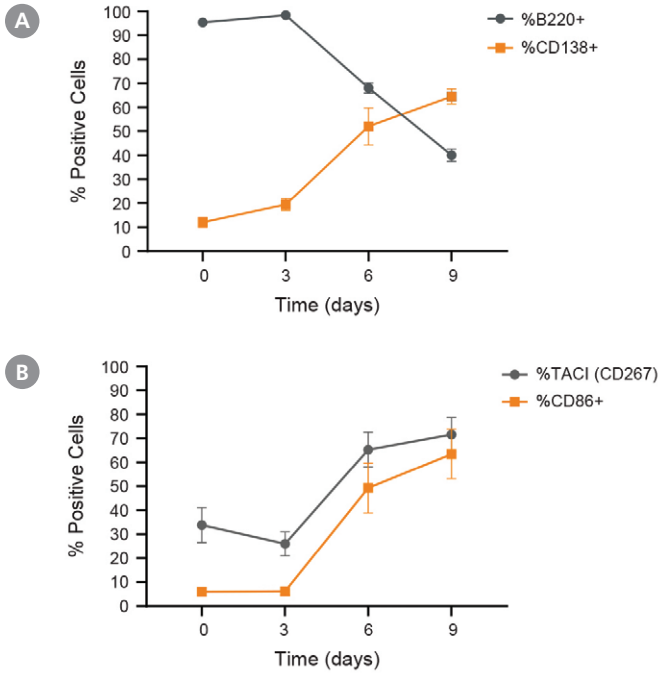


Figure 16. Maturation of Mouse B Cells with ImmunoCult™ Mouse B Cell Expansion Kit

B cells isolated from mouse spleen using EasySep™ Mouse Pan-B Cell Isolation Kit were cultured as described in Figure 14. Following staining using the protocol by Pracht et al. (Eur J Immunol, 2017), the expression of (A) B220 and CD138 and (B) TACI (CD267) and CD86 were analyzed by flow cytometry at several time points (data represents mean ± SEM; n = 8). An increase in CD86 cell surface expression indicates B cell activation; a decrease in B220 and an increase in CD138 and TACI cell surface expression indicate maturation of B cells to plasmablasts or plasma cells.

ImmunoCult™ B Cell Products

Product	Catalog #	Size
ImmunoCult™ Human B Cell Expansion Kit The kit includes Catalog #100-0646 and Catalog #10974	100-0645	1 kit
ImmunoCult™ Mouse B Cell Expansion Kit The kit includes Catalog #100-0646 and Catalog #100-1004	100-1003	1 kit
ImmunoCult™-XF B Cell Base Medium	100-0646	100 mL
ImmunoCult™-ACF Human B Cell Expansion Supplement	10974	2 mL
ImmunoCult™-ACF Mouse B Cell Expansion Supplement	100-1004	2 mL

Culture and Expand Natural Killer Cells

ImmunoCult™ for Human NK Cell Research

The ability to culture and expand NK cells has become a useful tool for researching the use of NK cells for adoptive immunotherapy in cancer patients as well as understanding basic NK cell biology. Optimize your culture conditions and expand human NK cells with high yields using the ImmunoCult™ NK Cell Expansion Kit. The components of this kit work together to provide you with a complete, easy-to-use culture system. Expanded NK cells can be harvested and used directly in your downstream applications.

The ImmunoCult™ NK Cell Expansion Kit is compatible with many of our other upstream and downstream products, including EasySep™ cell separation kits for isolating NK cells from blood or leukapheresis products.

Why Use ImmunoCult™ NK Cell Expansion Kit?

- Expand NK cells to obtain high cell yield and frequency
- Culture your cells in feeder- and serum-free conditions
- Obtain functional NK cells with cytotoxic potential
- Use cell culture reagents compatible with upstream cell isolation with EasySep™

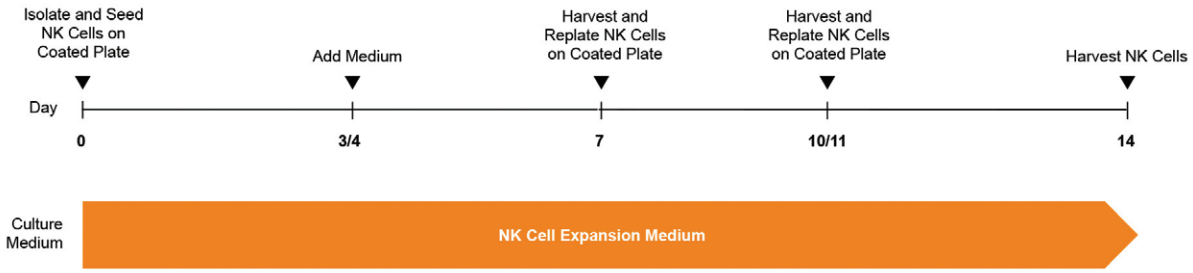


Figure 17. ImmunoCult™ NK Cell Expansion Protocol

Human natural killer (NK) cells are isolated from blood or leukapheresis using EasySep™ negative selection kit (e.g. EasySep™ Human NK Cell Isolation Kit; Catalog #17955). NK cells are cultured in ImmunoCult™ NK Cell Expansion Medium on plates coated with ImmunoCult™ NK Cell Expansion Coating Material. After 3 days, fresh medium is added to the culture. On Day 7, and again on Day 10 or 11, expanding NK cells are harvested and replated on freshly coated plates. Expanded NK cells can be harvested on Day 14 for use in downstream assays.

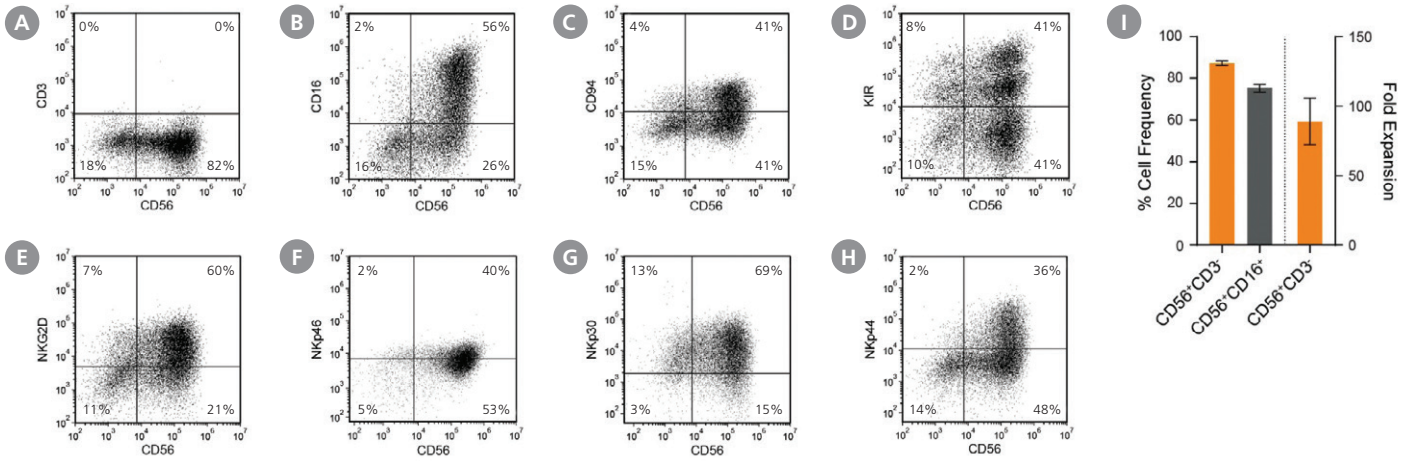


Figure 18. $CD56^+CD3^-$ NK Cells Expand Over 14 Days in Feeder- and Serum-Free Culture Conditions

Isolated human $CD56^+CD3^-$ NK cells were cultured using ImmunoCult™ NK Cell Expansion Kit for 14 days (see Figure 17). Cells were harvested and analyzed for expression of characteristic NK cell markers, including CD56, CD3, CD16, CD94, KIR, NKG2D, NKp46, NKp30, and NKp44 by flow cytometry. Staining for killer cell immunoglobulin-like receptor (KIR) molecules was performed using two different antibody clones, HP-MA4 and 180704, which recognize distinct KIR molecules. Dead cells were excluded by light-scatter profile and DRAQ7™ staining. (A - H) Representative flow cytometry plots. (I) The average frequencies of viable $CD56^+CD3^-$ and $CD56^+CD16^+$ NK cells on Day 14 were 87 ± 1% and 75 ± 2%, respectively. The average fold expansion of $CD56^+CD3^-$ cells was 89 ± 17. Results shown represent mean ± SEM (n = 34).

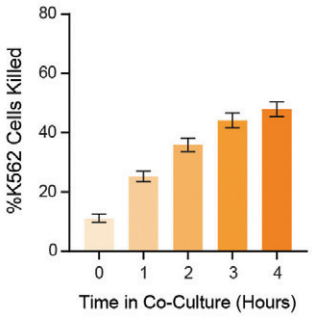


Figure 19. NK Cells Expanded Using ImmunoCult™ NK Cell Expansion Kit Are Functional, Killing K562 Cells in Co-Culture

Isolated CD56⁺CD3⁻ NK cells were expanded as described in Figure 17. Expanded NK cells were co-cultured with Incucyte® Cytolight Rapid Dye-labeled K562 cells at a 1:1 ratio of NK:K562 cells at 37°C for 4 hours. Incucyte® Caspase-3/7 Dye, a caspase-inducible dye, was added to the co-culture to detect caspase-induced apoptosis of the K562 cells. Images were obtained every hour using the Incucyte® imaging system and then analyzed to determine % killing (# apoptotic K562 cells ÷ # total labeled K562 cells). After 4 hours, an average of 48 ± 2.4% K562 cells were killed (n = 9). Data represents mean ± SEM.

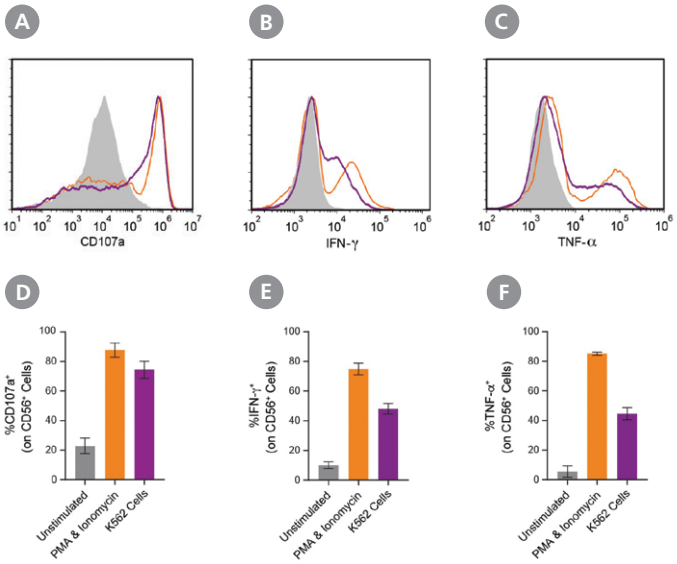


Figure 20. NK Cells Expanded Using ImmunoCult™ NK Cell Expansion Kit Degranulate and Produce Cytokines After Stimulation

Isolated CD56⁺CD3⁻ NK cells were expanded for 14 days (Figure 17). Expanded NK cells were left unstimulated (control) or were stimulated with either phorbol 12-myristate 13-acetate (PMA) and ionomycin or K562 cells at a ratio of 1:1 effector:target cells. CD107a antibody was added, and cultures were incubated at 37°C for 4 hours. After the first hour, Monensin and Brefeldin A were added. Cells were assessed for surface CD56, CD107a, and intracellular IFN-γ and TNF-γ expression by flow cytometry. (A-C) Representative histograms of CD107a, IFN-γ, and TNF-γ expression of unstimulated (gray filled), PMA and ionomycin-stimulated (orange), and K562-stimulated (purple) NK cell samples. (D) The average frequency of NK cells expressing surface CD107a, a marker of degranulation, was 23 ± 5% for the unstimulated control, 88 ± 5% after stimulation with PMA and ionomycin, and 74 ± 6% after stimulation with K562 cells. (E) The average frequency of NK cells expressing intracellular IFN-γ was 10 ± 2% for the unstimulated control, 75 ± 4% for cells stimulated with PMA and ionomycin, and 48 ± 4% for cells co-cultured with K562 cells. (F) The average frequency of NK cells expressing intracellular TNF-γ was 6 ± 4% for the unstimulated control, 85 ± 1% for cells stimulated with PMA and ionomycin, and 45 ± 4% for cells co-cultured with K562 cells. Data represents mean ± SEM (n = 6 - 13).

ImmunoCult™ Human NK Cell Products

Product	Catalog #	Size
ImmunoCult™ NK Cell Expansion Kit The kit includes Catalog #100-0712, Catalog #100-0714 and Catalog #100-0715	100-0711	1 kit
ImmunoCult™ NK Cell Base Medium	100-0712	500 mL
ImmunoCult™ NK Cell Expansion Supplement	100-0715	5 mL
ImmunoCult™ NK Cell Expansion Coating Material	100-0714	1.5 mL

PRODUCT SELECTION GUIDE
Human NK Cell Research
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PROTOCOL
CRISPR-Cas9 Genome Editing of Human NK Cells
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Differentiate Monocytes into Dendritic Cells

ImmunoCult™ for Dendritic Cell Research

Dendritic cells (DCs) are potent antigen-presenting cells and key regulators of the immune response. These cells are of great interest for research in cancer immunotherapy, vaccines, and infectious diseases. Advance your DC research by culturing and differentiating human monocytes into mature DCs with ImmunoCult™ Dendritic Cell Culture Kit, compatible with many of our other upstream and downstream products, including EasySep™ cell separation kits used to isolate monocytes for differentiation into dendritic cells.

Why Use ImmunoCult™ Dendritic Cell Culture Kit?

- Differentiate CD14+ monocytes into immature and subsequently mature DCs
- Reduce variability by using serum-free and animal component-free medium
- Achieve high yields of mature and functional DCs with the desired phenotype

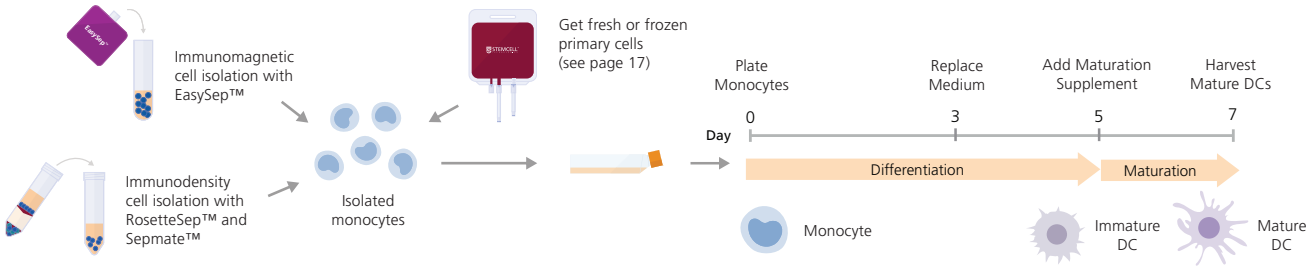

Figure 21. Protocol Diagram for the Generation of Dendritic Cells

Mature DCs were generated by culturing EasySep™ isolated monocytes at 1 x 10^6 cells/mL in ImmunoCult™-ACF Dendritic Cell Medium (Catalog #10987) with added ImmunoCult™-ACF Dendritic Cell Differentiation Supplement (Catalog #10988). On Day 3, the medium containing differentiation supplement was replaced and cells were incubated for 2 more days. On Day 5, without changing the medium, ImmunoCult™ Dendritic Cell Maturation Supplement (Catalog #10989) was added to the culture. On day 7, fully mature DCs were harvested for downstream applications.

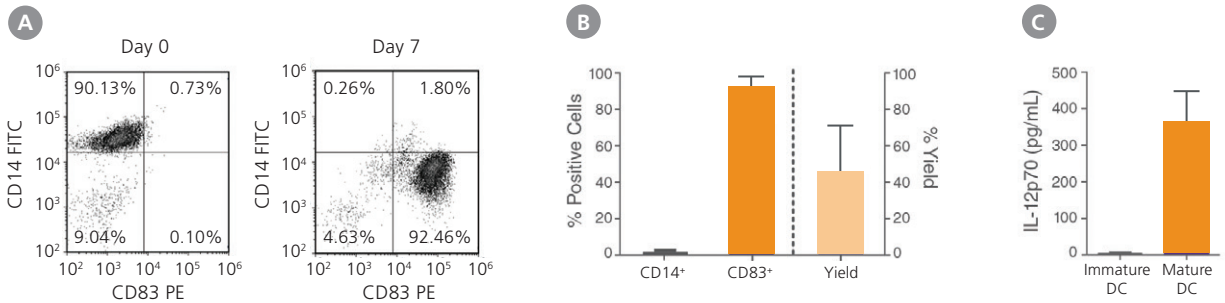

Figure 22. Mature DCs Generated with ImmunoCult™-ACF Dendritic Cell Medium and Supplements Show Desired Phenotype

Monocytes isolated using EasySep™ Human Monocyte Isolation Kit (Catalog #19359) were cultured and differentiated into mature DCs as described in Figure 21. (A) Representative flow cytometry plots of CD14 and CD83 expression in cells at Day 0 (monocytes) and at Day 7 (mature DCs). (B) The percentage of CD14 and CD83 expression in cells at Day 7 (mature DCs) was determined by flow cytometry. At Day 7, a total of 93 ± 5% of cells expressed the mature DC marker CD83 and only 1 ± 1% of cells still expressed the monocyte marker CD14 (mean ± SD; n = 39). The yield of mature DCs is expressed as the percentage of viable cells at Day 7 relative to the number of viable monocytes used for initial culture at Day 0. At Day 7, the yield of viable mature DCs corresponded to 45 ± 25% (mean ± SD; n = 39). (C) Immature DCs were cultured as described in Figure 21. At Day 5, cells were cultured with maturation supplement for 2 days (mature DCs) or without maturation supplement (immature DCs). Supernatant was collected on Day 7 and IL-12p70 levels were determined by ELISA. Concentrations of IL-12p70 in supernatants of mature and immature DCs were 361 ± 81 and 5 ± 2 pg/mL, respectively (mean ± SEM; n = 27).

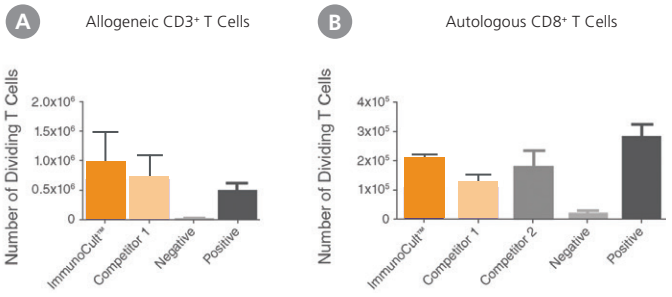

Figure 23. Mature DCs Generated with ImmunoCult™-ACF Dendritic Cell Medium and Supplements Induce T Cell Proliferation

Mature DCs generated with ImmunoCult™-ACF Dendritic Cell Medium and Supplements (ImmunoCult™) or other serum-free competitor media (competitor 1 and 2) and corresponding supplements when applicable (competitor 2), were cultured in ImmunoCult™-XF T Cell Expansion Medium with 1 x 10^5 CFSE-labeled (A) allogeneic $CD3^+$ T cells (MLR assay) or (B) autologous $CD8^+$ T cells (antigen-specific T cell response). (A) Cells were cultured at a DC:T cell ratio of 1:25. (B) Prior to culture with T cells, immature DCs were loaded with HLA Class I peptides derived from the human cytomegalovirus, Epstein-Barr Virus, and influenza virus (CEF peptide pool) and stimulated with maturation supplement for 2 days. Cells were cultured at a DC:T cell ratio of 1:4 or 1:10. (A,B) CFS-labeled T cells were incubated in media alone (negative control) or with ImmunoCult™ Human CD3/CD28 T Cell Activator (positive control). After 5 - 7 days in culture, the number of dividing T cells ($CD3^+CFSElo$) was assessed by flow cytometry (mean ± SEM) (A) n = 5 (B) n = 4 (competitor 1 and 2, n = 3). Mature DCs generated in ImmunoCult™-ACF Dendritic Cell Medium induced proliferation of allogeneic and antigen-specific T cells similar to DCs generated in either competitor media. Competitors 1 and 2, in no particular order, were CellGro DC Medium (CellGenix) and PromoCell DC Generation Medium DXF.

ImmunoCult™ Human DC Products

Product	Catalog #	Size
ImmunoCult™ Dendritic Cell Culture Kit The kit includes Catalog #10987, Catalog #10988, and Catalog #10989	10985	1 kit
ImmunoCult™-ACF Dendritic Cell Medium	10986	500 mL
	10987	100 mL
ImmunoCult™-ACF Dendritic Cell Differentiation Supplement	10988	1 mL
ImmunoCult™ Dendritic Cell Maturation Supplement	10989	0.5 mL

BROCHURE

Products for Dendritic Cell Research

www.stemcell.com/dcresearchflyer

Differentiate Monocytes into Macrophages

ImmunoCult™ for Macrophage Research

Macrophages are cells of the innate immune system, able to respond to infections or injury through phagocytosis as well as through the immunomodulatory cytokines they produce. Macrophages such as the "classically activated" M1 macrophages and "alternatively activated" M2 macrophages are cell types of great interest due to their role in immune regulation, tissue repair, and tumor biology. Advance your macrophage research and generate human macrophages with ImmunoCult™-SF Macrophage Medium.

This specialized medium has been developed for the in vitro culture of human monocytes and differentiation into macrophages when the appropriate cytokines and stimuli are added, providing the flexibility to differentiate human monocytes into M1 or M2a macrophages. ImmunoCult™-SF Macrophage Medium is compatible with many of our other upstream and downstream products, including cryopreserved monocytes (Catalog #70034), blood products, and RosetteSep™ and EasySep™ cell separation reagents.

Why Use ImmunoCult™-SF Macrophage Medium?

- Supports robust macrophage differentiation in serum-free conditions
- Obtain high yields of macrophages with the desired phenotype and function
- Generate M1 or M2 macrophages in a 6- or 8-day culture period

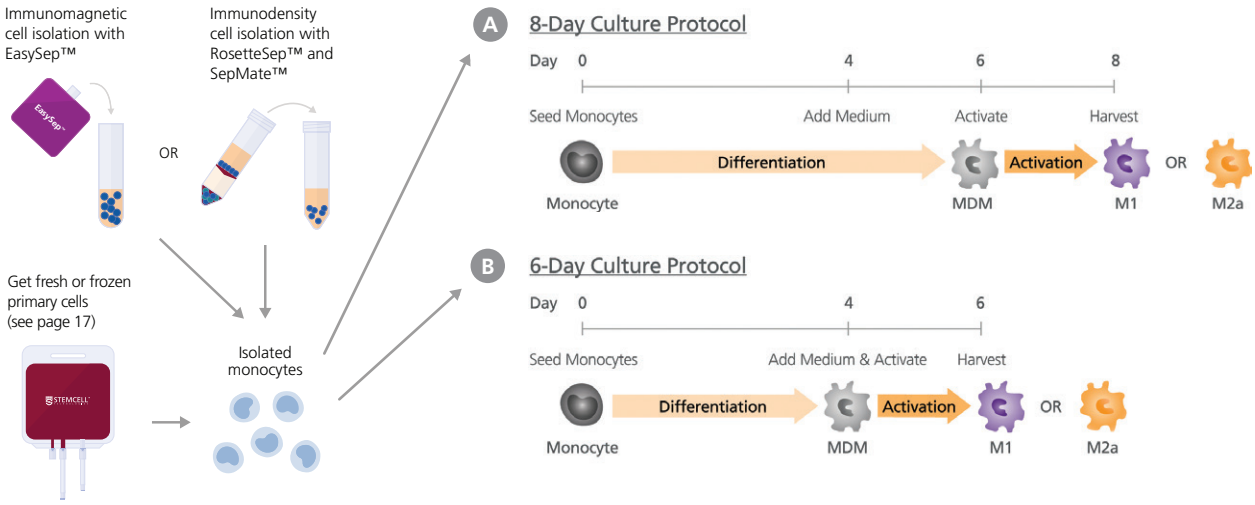

Figure 24. Protocol Diagram for the Generation of M1 or M2a Activated Macrophages

Generate monocyte-derived macrophages (MDM) from isolated monocytes using ImmunoCult™-SF Macrophage Differentiation Medium (ImmunoCult™-SF Macrophage Medium, Catalog #10961, with added Human Recombinant M-CSF, Catalog #78057). (A) If performing the 8-day protocol, fresh ImmunoCult™-SF Macrophage Differentiation Medium is added on Day 4 and specific macrophage activation can be achieved by adding appropriate stimuli on Day 6 (IFN-γ+LPS for M1 activation and IL-4 for M2a activation). On Day 8, fully mature M1 or M2a macrophages are ready for use in downstream applications. (B) If performing the 6-day protocol, macrophage activation can be done at the same time as the medium addition step on Day 4 and mature macrophages can be harvested on Day 6.

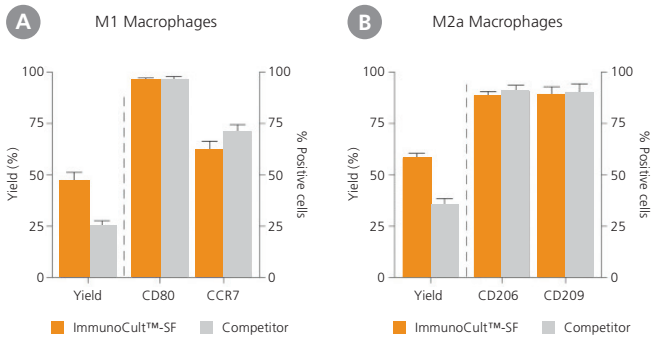

Figure 25. ImmunoCult™-SF Supports Greater M1 and M2a Macrophage Yields Than Competitor's Serum-Free Medium

Monocytes were cultured in ImmunoCult™-SF Macrophage Medium or a competitor's serum-free macrophage medium and differentiated into macrophages using an 8-day protocol as shown in Figure 24. On Day 8, macrophages were harvested, counted, and analyzed by flow cytometry to assess the expression of macrophage markers CD80, CCR7, CD206, and CD209. (A) M1 macrophages were $CD80^+CCR7^+$ whereas (B) M2a macrophages showed a $CD206^+CD209^+$ phenotype. Macrophage yields are expressed as a percentage of total viable cells at Day 8 relative to the initial number of monocytes at Day 0. Macrophage yields were significantly higher in ImmunoCult™-SF than in competitor's serum-free medium ($p < 0.05$, paired t-test; mean ± SEM; n = 18 - 19).

ImmunoCult™ Human Macrophage Products

Product	Catalog #	Size
ImmunoCult™-SF Macrophage Medium	10961	250 mL

WALLCHART

Antigen Processing and Presentation

www.stemcell.com/apcwallchart

BROCHURE

Products for Macrophage Research

www.stemcell.com/macrophageresearchflyer

Differentiate hPSCs into Immune Cells

The ability to differentiate human pluripotent stem cells (hPSCs) into immune cells is a useful tool when developing adoptive immunotherapies for cancer patients as well as when seeking a better understanding of the biology of these cells. STEMdiff™ and StemSpan™ immune kits facilitate the differentiation of human pluripotent stem cells (hPSCs) or CD34$^+$ cells into immune cells—without the need for stromal cells or serum.

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Expand your research into the development of T and NK lineage cells from normal or leukemic human hematopoietic stem and progenitor cells (HSPCs) with StemSpan™ media and supplement kits:

- Differentiate T cells or NK cells from CD34$^+$ stem and progenitor cells without stromal cells or serum
- Obtain high yields of CD4$^+$CD8$^+$ DP T cells or CD56$^+$ NK cells per input CD34$^+$ cell

For more information, please visit **www.StemSpan.com**.

STEMdiff™ for T Cell and NK Cell Research

Consistently differentiate embryonic stem (ES) and induced pluripotent stem (iPS) cells into T cells or NK cells with high yield and viability:

- Eliminate variation introduced by serum and stromal cell lines by using serum- and feeder-free conditions
- Produce approximately 230 CD56$^+$ NK cells or 60 CD4$^+$CD8$^+$ double-positive (DP) T cells per input hPSC-derived CD34$^+$ cell
- Generate uniform embryoid body (EB) aggregates with AggreWell™
- Avoid extra passaging steps required with stromal cell-based cultures

For more information, please visit **www.stemcell.com/STEMdiff-T** or **www.stemcell.com/STEMdiff-NK**.

STEMdiff™ for Monocyte Research

Reliably generate millions of CD14$^+$ monocytes from embryonic stem (ES) and induced pluripotent stem (iPS) cell lines:

- Generate up to 7 million CD14$^+$ monocytes per 6-well plate at peak harvest in just 14 - 23 days
- Eliminate variation introduced by serum and feeder cells by using serum- and feeder-free conditions
- Produce monocytes in a simple monolayer culture for easier harvest of suspended cells
- Achieve robust generation of monocytes across multiple ES and iPS cell lines

The STEMdiff™ Monocyte Kit generates hPSC-derived monocytes that can be further differentiated into dendritic cells or macrophages using the ImmunoCult™ Dendritic Cell Culture Kit or ImmunoCult™-SF Macrophage Medium, respectively.

For more information, please visit **www.stemcell.com/STEMdiff-Monocyte**.

TECH BULLETIN

Generation of Monocytes from Human Pluripotent Stem Cells

www.stemcell.com/stemdiffmonocytes-tb

TECH BULLETIN

Generation of T Cells from Human Pluripotent Stem Cells

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TECH BULLETIN

Generation of Natural Killer Cells from Human Pluripotent Stem Cells

www.stemcell.com/stemdiffnk-tb

StemSpan™ Products

Product	Catalog #	Size	Applications
StemSpan™ NK Cell Generation Kit	09960	1 kit	For expansion and differentiation of human CD34+ hematopoietic progenitor cells to NK cells
StemSpan™ T Cell Generation Kit	09940	1 kit	For expansion and differentiation of human CD34+ hematopoietic progenitor cells to T cells
StemSpan™ Leukemic Cell Culture Kit	09720	1 kit	For culture, expansion, and drug screening of chronic and acute myeloid leukemia cells

STEMdiff™ Products

Product	Catalog #	Size	Applications
STEMdiff™ T Cell Kit	100-0194	1 kit	For expansion and differentiation of hPSCs to T cells
STEMdiff™ NK Cell Kit	100-0170	1 kit	For expansion and differentiation of hPSCs to NK cells
STEMdiff™ Monocyte Kit	05320	1 kit	For expansion and differentiation of hPSCs to monocytes
STEMdiff™ Microglia Differentiation Kit	100-0019	1 kit	For differentiation of microglia precursors from hPSC-derived hematopoietic progenitor cells
STEMdiff™ Microglia Maturation Kit	100-0020	1 kit	For maturation of microglia from hPSC-derived microglia precursors

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Complete your workflow with primary cells, gene editing products, cytokines, cryopreservation media, cultureware, and more.

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Using human primary cells instead of immortalized cell lines increases the physiological relevance of data obtained from cell culture systems. Primary cells are increasingly recognized for their importance in the study of biological processes, disease progression, and drug development. Choose from a wide range of fresh or cryopreserved human primary cells isolated from peripheral blood, cord blood, bone marrow, and mobilized peripheral blood.[1,2]

Cryopreserved immune and progenitor cells isolated from full-size leukopaks (leukapheresis preparations) or entire umbilical cords are ready to use upon receipt. For users requiring fresh, unprocessed tissue samples, whole peripheral blood, whole bone marrow, leukocyte reduction system (LRS) cones, and leukopaks are also available.[3]

For a complete listing of primary cells products, including mobilized peripheral blood products and cultured cells, please visit **www.stemcell.com/primarycells**.

Why Use STEMCELL's Human Primary Cells?

- Choose cells that are more physiologically representative of cells in vivo
- Access donor samples collected using regulatory authority-approved consent forms and protocols
- Request custom products for non-standard cell types or collections with specific requirements
- Reserve large numbers of cryopreserved cells and start experiments on your schedule with cells you've already tested
- Reduce time spent collecting and culturing primary cells

1. *Certain cryopreserved products are only available in select territories. Please contact Product and Scientific Support (techsupport@stemcell.com) for further information.*
2. *Fresh products currently available in the United States and Canada (excluding Quebec). Please contact Product and Scientific Support (techsupport@stemcell.com) for further information.*
3. *LRS cone, leukopak (LP), whole blood (WB), and bone marrow (BM) donors are screened for HIV-1, HIV-2, hepatitis B, and hepatitis C. Cryopreserved LP, WB, and BM: If the donor has tested negative within 90 days prior to donation, the product will be shipped with the negative test result and date of most recent viral testing on the Certificate of Analysis (CoA). Fresh LRS cone, 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 most recent viral testing on the 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 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, and within 4 - 7 business days in the case of fresh LRS Cones). Cord blood (CB) donor screening: Maternal blood samples and/or samples of the donated CB are tested for HIV-1, HIV-2, hepatitis B, and hepatitis C. Cryopreserved CB: Products with negative test results are shipped with the CoA.*

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For more information, please visit **www.stemcell.com/ThawSTAR-prod**.

Product	Catalog #	Size
ThawSTAR® CFT2 Automated Thawing System	100-0650	1 unit
ThawSTAR® CB Automated Thawing System	100-1151	1 unit

Why Use ThawSTAR® Automated Thawing Systems?

- Obtain reproducible thawing profiles using a standardized thawing method
- Eliminate risk of contamination from water bath-based thawing techniques
- Maximize viability and function of cryo-sensitive cells
- Convenient and compact format
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Automated Thawing System

ThawSTAR® CB Automated Thawing System

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For more information, please visit **www.stemcell.com/ArciTect**.

TECH BULLETIN

Genome Editing of Human Primary T Cells Using CRISPR-Cas9

www.stemcell.com/tcell-editing

VIDEO

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WALLCHART

Frequencies of Human Cell Types in Blood-Related Sources

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Recombinant Cytokines

Product	Catalog #		
Recombinant Cytokine	Human	Mouse	Rat
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G-CSF[1,2]	78012	78014	--
M-CSF[1,2]	78057	78059	78117
IFN-β	78113	--	--
IFN-γ[1]	78020	78021	78114
TNF-α[1]	78068	78069	78124
TNF-β	78125	--	--
TNF-receptor 1	78126	--	--
GRO-beta (CXCL2)	78112	--	--
MIP-3α (CCL20)	78118	--	--
TRAIL	--	78122	--
IL-1α[1]	78115	78129	--
IL-1β[1]	78034	78035	--
IL-2[1,2]	78036	78081	--
IL-3[1,2]	78040	78042	78181
IL-4[1,2]	78045	78047	--
IL-5[1]	78048	78049	--
IL-6[1]	78050	78052	--
IL-7[1]	78053	78054	--
IL-10[1,2]	78024	78079	--
IL-11[1]	78025	78026	--
IL-12	78027	78028	--
IL-13	78029	78030	--
IL-15	78031	78080	--
IL-17A	78032	78033	--
IL-21	78082	78116	--
IL-22	78038	78039	--
IL-33	78043	78044	--

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Cryopreservation Media

Product	Catalog #	Size
CryoStor® CS10	07930 07931 07940 07952 07955 07959	100 mL 5 x 16 mL vials 1000 mL bag 16 x 10 mL 100 mL bag 5 x 10 mL
CryoStor® CS5	07933 07949 07953	100 mL 5 x 10 mL 100 mL bag
CryoStor® CS2	07932	100 mL
CryoStor® CSB	100-0237 100-0238 100-0239	100 mL 500 mL 1000 mL

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Complete your workflow with a range of cultureware, including trusted brands like Corning® and Axygen®, that are compatible with our cell isolation kits, cell culture media, genome editing tools, and molecular biology reagents.

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