

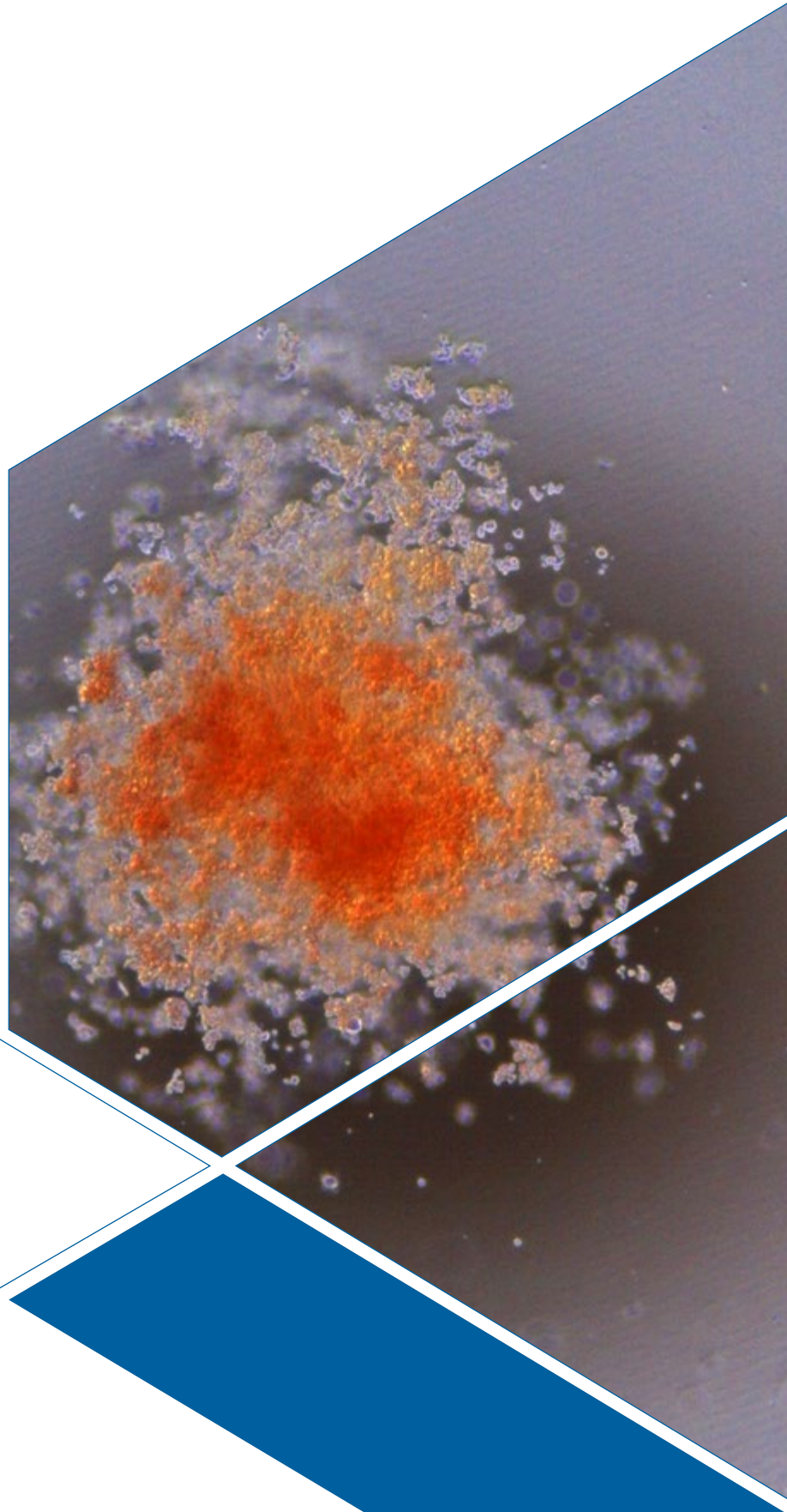
# Hematopoietic Stem Cells

ISOLATE & CULTURE

VERIFY

DIFFERENTIATE

INVESTIGATE



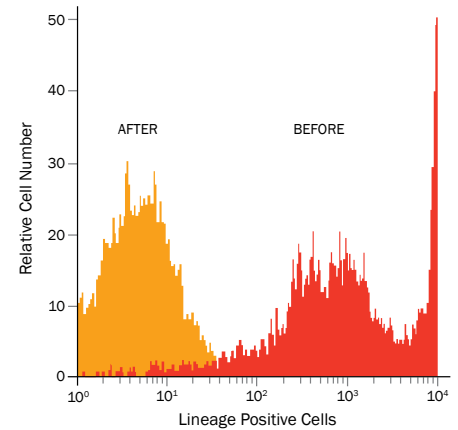
# ISOLATE AND CULTURE

## Mouse Hematopoietic Cell Lineage Depletion Kit (Catalog # MAGM209)

Both positive and negative selection methods can be used to generate cell suspensions with high purity. However, cells enriched by positive selection can generate cells that are labeled with antibodies and/or beads. These modifications can introduce experimental variability and can invalidate the use of certain antibodies in downstream applications such as flow cytometry. To efficiently obtain untouched cells of high purity by negative selection, R&D Systems offers the MagCelect™ Mouse Hematopoietic Cell Lineage Depletion Kit. The MagCelect Mouse Hematopoietic Cell Lineage Depletion Kit uses a magnet and a cocktail of antibodies to remove unwanted, lineage-positive cells from a cell suspension. Non-lineage committed mouse hematopoietic cells isolated with the kit are untouched by antibodies or magnetic particles and can be used for any downstream application.

### Features

- **Convenient:** can be used in conjunction with our MagCelect™ Cell Enrichment System
- **Specific:** use of several antibodies increases purity
- **Fewer variables:** negatively selected cells are untouched by beads or antibodies
- **Thorough:** depletes T cells, B cells, NK cells, monocytes/macrophages, granulocytes, and erythrocytes
- **Complete:** includes biotinylated depletion antibody cocktail, MagCelect streptavidin ferrofluid, blocking buffer, and wash buffer
- **Robust:** each kit processes up to  $1 \times 10^9$  cells



**Depletion of Lineage-committed Hematopoietic Cells.** Lineage marker reactivity on BALB/c bone marrow (BM) cells processed with the MagCelect Mouse Hematopoietic Cell Lineage Depletion Kit (Catalog # MAGM209). The histograms show the reactivity of BM cells labeled with the cocktail of biotinylated antibodies (Rat Anti-Mouse CD5, CD11b, B220/CD45R, Gr-1/Ly-6G, and TER-119) included in the kit both before (red histogram) and after (orange histogram) magnetic depletion. Lineage marker reactivity was detected using Streptavidin-PE.

## Individual Anti-Mouse Monoclonal Antibodies for Hematopoietic Lineage Depletion

### Features

- **Reliable:** efficiently bind to lineage-committed bone marrow-derived cells
- **Flexible:** can be used with magnetic separation systems or with flow cytometry cell sorting for enrichment of uncommitted HSCs
- **Validated:** antibodies are optimized to bind to  $1 \times 10^9$  bone marrow-derived cells

Product	Description	Catalog #
Mouse B220/CD45R	Monoclonal Rat IgG <sub>2a</sub> (Clone RA3-6B2)	MLDP7
Mouse CD3	Monoclonal Rat IgG <sub>2b</sub> (Clone 17A2)	MLDP1
Mouse CD4	Monoclonal Rat IgG <sub>2b</sub> (Clone GK1.5)	MLDP2
Mouse CD5	Monoclonal Rat IgG <sub>2a</sub> (Clone 53-7.3)	MLDP3
Mouse CD8 $\alpha$	Monoclonal Rat IgG <sub>2a</sub> (Clone 53-6.7)	MLDP4
Mouse Integrin $\alpha$ M/CD11b/MAC-1	Monoclonal Rat IgG <sub>2b</sub> (Clone M1/70)	MLDP5
Mouse Gr-1/Ly-6G	Monoclonal Rat IgG <sub>2b</sub> (Clone RB6-8C5)	MLDP6
Mouse TER-119 Erythroid Antigen	Monoclonal Rat IgG <sub>2b</sub> (Clone TER-119)	MLDP8

# Cell Culture Reagents for HSC and Derivative Cell Expansion and Differentiation

Hematopoiesis is regulated, in part, by extrinsic growth factors and cytokines which activate intracellular signaling pathways that influence HSC multipotency, proliferation, and lineage commitment. These signaling pathways can be modulated by naturally occurring and synthetic small molecules. For example, compounds can be targeted to a specific protein within a pathway, and the cellular response can often be controlled by subtle changes in compound concentration. Given that many small molecules act on a desired pathway, researchers may enhance stem cell proliferation and direct cell fate decisions by manipulating specific signal transduction pathways.

## Individual Cytokines and Growth Factors

### Features

- **Reliable:** minimal lot-to-lot variability
- **Active:** biological activity is measured with an appropriate biological system
- **Pure:** purity is typically >95%
- Many available as GMP-grade proteins



Recombinant Protein	Catalog #			ProDots Proteins, Catalog #	GMP Grade, Catalog #
	Human	Mouse	Rat	Human	Human
Angiopoietin-like Protein 5/ANGPTL5	6675-AN				
CD117/c-kit	332-SR	1356-SR			
Flt-3 Ligand	308-FK	427-FL		PRD308	308-GMP
GM-CSF	215-GM	415-ML	518-GM	PRD215	215-GMP
IL-2	202-IL	402-ML	502-RL	PRD202	202-GMP
IL-3	203-IL	403-ML	2524-RL	PRD203	203-GMP
IL-4	204-IL	404-ML	504-RL	PRD204	204-GMP
IL-7	207-IL	407-ML	7857-RL		207-GMP
IL-11	218-IL	418-ML			
IL-15	247-ILB	447-ML			247-GMP

Recombinant Protein	Catalog #			ProDots Proteins, Catalog #	GMP Grade, Catalog #
	Human	Mouse	Rat	Human	Human
M-CSF	216-MC	416-ML		PRD216	216-GMP
Notch-1 Fc Chimera	3647-TK	5267-TK	1057-TK		
Notch-2 Fc Chimera	3735-NT	5196-NT	1190-NT		
Notch-3 (aa 40-460) Fc Chimera	1159-NT	1308-NT			
SCF/c-kit Ligand	255-SC	455-MC	6998-SC		255-GMP
Thrombopoietin/Tpo	288-TPN	488-TO	7864-TP		
TNF- $\alpha$	210-TA	410-MT	510-RT	PRD220	210-GMP
VEGF 165	293-VE			PRD293	293-GMP
Wnt-3a	5036-WN	1324-WN			5036-GMP

## Mouse Hematopoietic Stem Cell Expansion Cytokine Panel (Catalog # SMPK9)

### Features

- Contains 100  $\mu$ g of Recombinant Mouse Flt-3 Ligand, Tpo, and SCF
- Optimized for efficient HSC expansion

## Tocris® Small Molecules

### Features

- Increase differentiation efficiency
- Minimize the use of animal-derived factors
- Gain temporal control of differentiation pathways

Molecule	Use in Stem Cell Research	Catalog #
AMD 3100 octahydrochloride	Mobilization of HSCs; HSC expansion	3299
BIO-acetoxime	HSC expansion	3874
CH 223191	HSC expansion	3858
CHIR 99021	HSC expansion	4423
DiD perchlorate	HSC stain	5702
16, 16-Dimethyl Prostaglandin E2	HSC expansion	4027
Diprotin A	Enhances HSC viability after harvesting	6019
NSC 23005 sodium	HSC expansion	5779
Prostaglandin E <sub>2</sub>	HSC expansion and homing; increases HSC engraftment	2296
SB 431542	HSC expansion	1614
Shz 1	Induces differentiation in M-PBMCs	4923
SW 033291	Promotes hematopoiesis	5759
Tocriscreen Stem Cell Toolbox	High-throughput and high content screening	5060

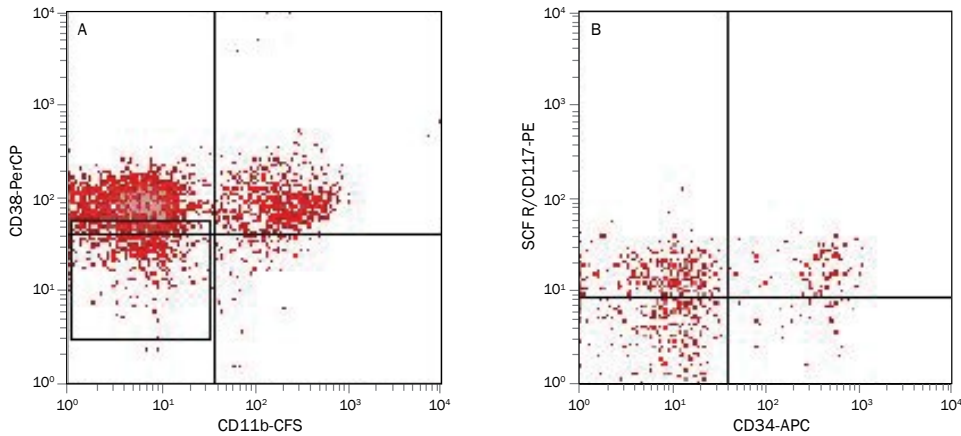
# VERIFY

## Human Hematopoietic Progenitor Cell Multi-Color Flow Cytometry Kit (Catalog # FMC019)

Researchers use different techniques to isolate, culture, and differentiate hematopoietic progenitor cells. Variations in experimental approaches as well as differences in the starting cell population may account for experimental variability and contradictory data that have been published in the stem cell field. One way to minimize experimental variability is to clearly define the starting cell population by using R&D Systems Human Hematopoietic Progenitor Cell Multi-Color Flow Cytometry Kit.

### Features

- Verifies hematopoietic progenitor cell multipotency
- Defines the starting population to reduce experimental variation
- Simultaneously detects or 4 established multipotency markers

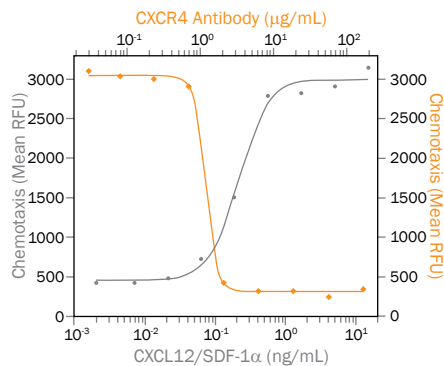


**Verification of Human Umbilical Cord-derived Hematopoietic Progenitor Cell Identity using Multi-Color Flow Cytometry.** Human umbilical cord blood cells were stained using reagents supplied in the Human Hematopoietic Progenitor Cell 4-Color Flow Kit (Catalog # FMC019). Cells negative for CD11b and negative/low for CD38 (boxed area in A) were gated and assessed for positive expression of CD34 and SCF R/CD117 (upper right quadrant in B). Quadrants were set based on isotype controls.

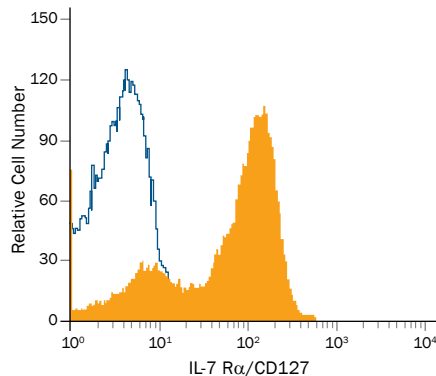
## Individual Antibodies for HSC Characterization

### Features

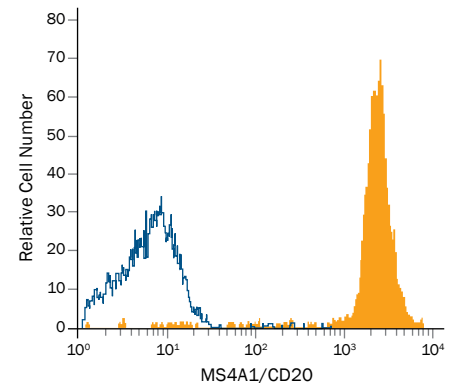
- **Reliable:** undergo rigorous quality testing to ensure lot-to-lot consistency and outstanding performance
- **Specific:** tested for cross-reactivity with related molecules by direct ELISA
- Neutralizing antibodies are tested to ensure low endotoxin levels
- Sample size vials available



**Chemotaxis Induced by CXCL12/SDF-1 $\alpha$  and Neutralized by a CXCR4 Antibody.** Recombinant Human/Feline/Rhesus Macaque CXCL12/SDF-1 $\alpha$  (Catalog # 350-NS) chemoattracts the BaF3 mouse pro-B cell line expressing human CXCR4 in a concentration-dependent manner (gray line). Cells that migrated through to the lower chemotaxis chamber were measured using Resazurin (Catalog # ARO02). Chemotaxis elicited by 1 ng/mL Recombinant Human/Feline/Rhesus Macaque CXCL12/SDF-1 $\alpha$  is neutralized (orange line) by increasing concentrations of a Mouse Anti-Human CXCR4 Monoclonal Antibody (Catalog # MAB173).



**Detection of IL-7 R $\alpha$ /CD127 by Flow Cytometry.** Human peripheral blood lymphocytes were stained with an APC-conjugated Mouse Anti-Human IL-7 R $\alpha$ /CD127 Monoclonal Antibody (Catalog # FAB306A; filled histogram) or an APC-conjugated Mouse IgG<sub>1</sub> Isotype Control (Catalog # IC002A; open histogram).



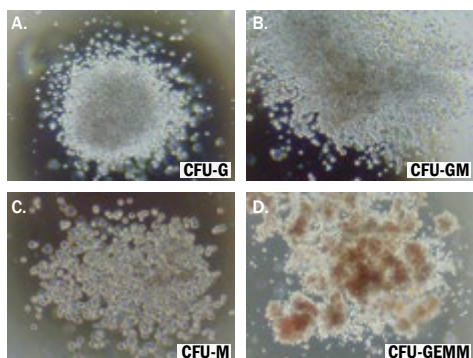
**Detection of MS4A1/CD20 by Flow Cytometry.** Human B cells were stained with a Fluorescein-conjugated Mouse Anti-Human MS4A1/CD20 Monoclonal Antibody (Catalog # FAB4225F; filled histogram) or a Fluorescein-conjugated Mouse IgG<sub>1</sub> Isotype Control (Catalog # IC002F; open histogram).

# DIFFERENTIATE

## R&D Systems® Methylcellulose-Based Reagents

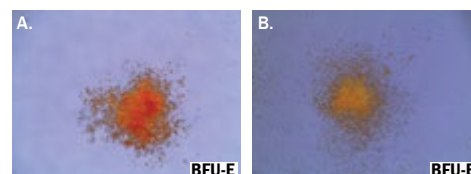
### Features

- **Flexible:** available as complete media or incomplete media that can be supplemented with user-defined cytokines and growth factors
- **Reliable:** high lot-to-lot consistency decreases variation
- **Excellent Clarity:** optical clarity facilitates easier colony identification
- **Rich Color:** BFU-E colonies appear red in color to facilitate colony identification



#### Human Hematopoietic Colony Formation Using the Methylcellulose-based Colony Forming Cell Assay.

**A.** Colony forming unit-granulocyte (CFU-G) are clonogenic progenitors of granulocytes that give rise to a homogeneous population of eosinophils, basophils, or neutrophils. **B.** Colony forming unit-granulocyte, macrophage (CFU-GM) are progenitors that give rise to colonies containing a heterogeneous population of macrophages and granulocytes. The morphology is similar to the CFU-M and CFU-G descriptions. **C.** Colony forming unit-macrophage (CFU-M) are clonogenic progenitors of macrophages that give rise to a homogeneous population of macrophages. **D.** Colony forming unit-granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM) are multi-lineage progenitors that give rise to granulocyte, erythroid macrophage and megakaryocyte lineages, as the name indicates.



#### Mouse Colony Forming-Cells Assay on Bone Marrow Cells.

The colony forming cell assay was performed on mouse bone marrow cells cultured for 8 days using either Mouse Methylcellulose Complete Media (Catalog # HSC007) (A) or media from a competitor (B). The colony of burst forming unit erythroid cells (BFU-E) cultured in the Mouse Methylcellulose Complete Media (A) displayed a more robust red color to aid in its identification than is seen with the competitor media (B).

Human Methylcellulose Media		
Product	Cytokines Included	Catalog #
Methylcellulose Stock Solution	None	HSC001
Base Media	None	HSC002
Serum-Free Base Media	None	HSC002SF
StemXVivo® Methylcellulose Concentrate	None	HSC011
Complete Media	Epo, GM-CSF, IL-3, SCF	HSC003
Complete Media without Epo	GM-CSF, IL-3, SCF	HSC004
Enriched Media	Epo, G-CSF, GM-CSF, IL-3, IL-6, SCF	HSC005
Serum-Free Enriched Media	Epo, G-CSF, GM-CSF, IL-3, IL-6, SCF	HSC005SF
Serum-Free Enriched Media without Epo	G-CSF, GM-CSF, IL-3, IL-6, SCF	HSC010SF

Mouse Methylcellulose Media		
Product	Cytokines Included	Catalog #
Methylcellulose Stock Solution	None	HSC001
Mouse Methylcellulose	None	HSC006
StemXVivo® Methylcellulose Concentrate	None	HSC011
Complete Media	Epo, IL-3, IL-6, SCF	HSC007
Complete Media Without Epo	IL-3, IL-6, SCF	HSC008
Complete Media for Pre-B Cells	IL-7	HSC009

Rat Methylcellulose Media		
Product	Cytokines Included	Catalog #
Methylcellulose Stock Solution	None	HSC001
StemXVivo® Methylcellulose Concentrate	None	HSC011
Methylcellulose Complete Media Without Epo	GM-CSF, IL-3, SCF	HSC012



# Immune Cell Differentiation and Expansion Kits

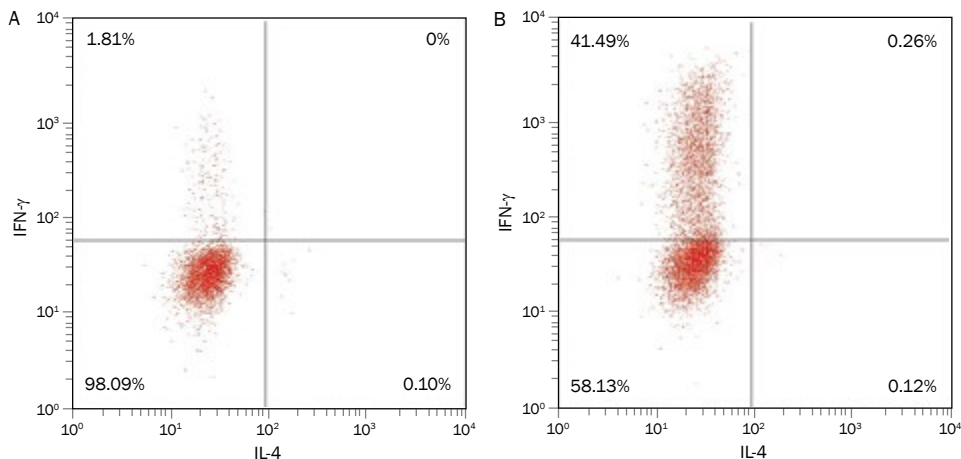
R&D Systems offers CellXVivo™ kits containing our high quality cytokines to differentiate and expand mature lymphoid and myeloid immune cells from enriched populations of human peripheral blood mononuclear cells (PBMCs). *Ex vivo* differentiation of leukocytes into immune effector cells limits the variability that often occurs *in vivo*. The ability to expand immune cell populations *ex vivo* provides increased numbers of these cells for downstream research.

## Features

- Optimized cocktails of high quality bioactive cytokines to induce reliable differentiation or expansion
- Differentiation kits yield highly enriched populations of differentiated cells
- Validated and straightforward procedures

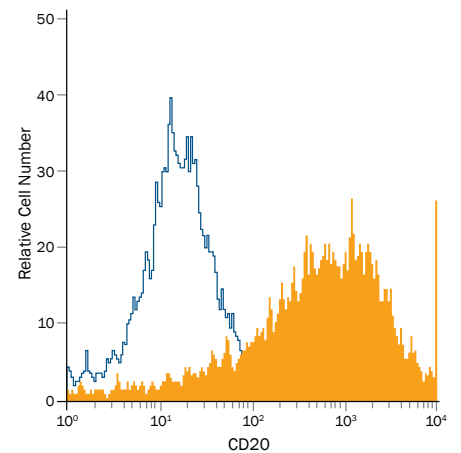
CellXVivo Kit	Species	Description	Catalog #
B Cell Expansion Kit	Human	For the <i>ex vivo</i> expansion of B cells	CDK005
CIK Cell Expansion Kit	Human	Expands human CD3 <sup>+</sup> CD56 <sup>+</sup> cytokine-induced killer cells from PBMCs	CDK014
NK Cell Expansion Kit	Human	Expands human CD3 <sup>-</sup> CD56 <sup>+</sup> natural killer cells from PBMCs	CDK015
Th1 Cell Differentiation Kit	Human	Differentiates CD4 <sup>+</sup> lymphocytes into mature Th1 effector cells	CDK001
	Mouse		CDK018
Th2 Cell Differentiation Kit	Human	Differentiates CD4 <sup>+</sup> lymphocytes into mature Th2 effector cells	CDK002
	Mouse		CDK019
Th17 Cell Differentiation Kit	Human	Differentiates CD4 <sup>+</sup> human lymphocytes into mature Th17 effector cells	CDK003C
	Mouse		CDK017
Treg Cell Differentiation Kit	Human	Differentiates CD4 <sup>+</sup> human lymphocytes into CD25 <sup>+</sup> FoxP3 <sup>+</sup> regulatory T cells	CDK006
	Mouse		CDK007
Monocyte-derived Dendritic Cell Differentiation Kit	Human	Differentiate CD14 <sup>+</sup> monocytes into monocyte-derived dendritic cells	CDK004
	Mouse	Differentiate mouse bone marrow cells into dendritic cells	CDK008
M1 Macrophage Differentiation Kit	Human	Differentiate CD14 <sup>+</sup> monocytes into M1 Macrophages	CDK012
M2 Macrophage Differentiation Kit	Human	Differentiate CD14 <sup>+</sup> monocytes into M2 Macrophages	CDK013

## Th1 Differentiation



**Verification of Th1 Cell Identity using Flow Cytometry.** Human peripheral blood naïve CD4<sup>+</sup> T cells without (A) and with (B) a 5 day differentiation using the reagents included in the CellXVivo™ Human Th1 Cell Differentiation Kit (Catalog # CDK001). The cells were stained with an APC-conjugated Mouse Anti-Human IFN- $\gamma$  Monoclonal Antibody (Catalog # IC285A) and a PE-conjugated Mouse Anti-Human IL-4 Monoclonal Antibody (Catalog # IC204P). Control cultures were used to place the quadrants.

## B Cell Expansion

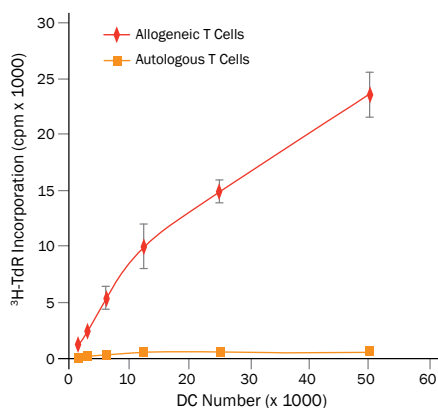


**Detection of CD20 in Human B Cells.** Human B cells were expanded for 5 days using reagents included in the CellXVivo™ Human B Cell Expansion Kit (Catalog # CDK005). The cells were labeled with a PE-conjugated Mouse Anti-Human CD20 Monoclonal Antibody (Catalog # FAB4225P; filled histogram) or a PE-conjugated Mouse IgG1 Isotype control (Catalog # IC002P; open histogram).

## StemXVivo® Serum-Free Dendritic Cell Base Media (Catalog # CCM003)

### Features

- Pre-optimized media designed and validated for dendritic cell culture
- High lot-to-lot consistency decreases variation
- Can be supplemented with user-defined cytokines and growth factors

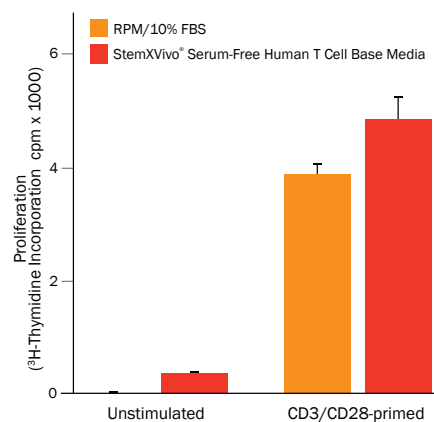


**Mature Monocyte-derived Dendritic Cells Induce Proliferation of Allogeneic T Cells.** CD14<sup>+</sup> monocytes were cultured for seven days in Human StemXVivo® Serum-free Dendritic Cell Base Media (Catalog # CCM003) supplemented with Recombinant Human GM-CSF (Catalog # 215-GM), Recombinant Human IL-4 (Catalog # 204-IL) and Gentamycin. The cells were subsequently treated with LPS for an additional 48 hours to induce dendritic cell maturation. Graded doses of mature monocyte-derived dendritic cells were incubated with  $1 \times 10^5$  autologous or allogeneic CD3<sup>+</sup> T cells for five days. <sup>3</sup>H-thymidine (<sup>3</sup>H-TdR) was added to the culture for the final 18 hours and T cell proliferation was measured using a scintillation counter. Results are presented as the mean cpm obtained from three experiments.

## StemXVivo® Serum-Free Human T Cell Base Media (Catalog # CCM010)

### Features

- Supports T lymphocyte expansion as well as or better than RPMI containing FBS
- Defined media decreases experimental variation
- High lot-to-lot consistency increases reproducibility



**Proliferative Response of Cultured CD3/CD28-Primed T cells in Human StemXVivo® Serum-Free T Cell Base Media.**  $1 \times 10^5$  purified CD3<sup>+</sup> T cells were cultured for five days in StemXVivo® Serum-Free T Cell Base Media (Catalog # CCM010) or with RPMI supplemented with 10% Fetal Bovine Serum (RPMI/10% FBS). The purified CD3<sup>+</sup> T cells were cultured on 96 well microplates coated with Mouse Anti-Human CD3  $\epsilon$  (Clone UCHT1; Catalog # MAB100) and Goat Anti-Human CD28 Antigen Affinity-purified Polyclonal Antibody. [<sup>3</sup>H]-thymidine was added for the final 18 hours. Cells were harvested and the incorporation of [<sup>3</sup>H]-thymidine was measured using a beta-scintillation counter. Results are presented as the mean  $\pm$  standard deviation of samples run in triplicate.

## INVESTIGATE

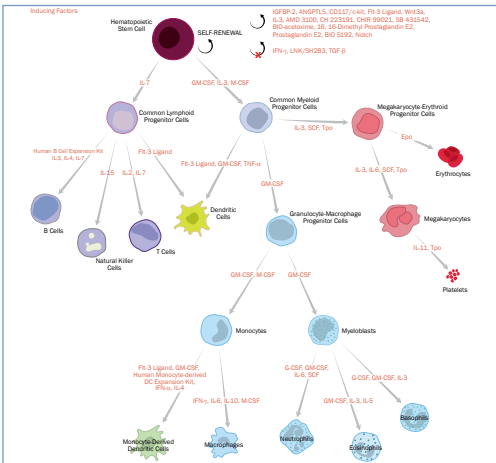
### Proteome Profiler™ Human Soluble Receptor Array, Hematopoietic Panel (Catalog # ARY011)

The R&D Systems® Human Soluble Receptor Array, Hematopoietic Panel is a rapid, sensitive, and economical tool used to simultaneously detect the relative levels of 105 different soluble receptors expressed and released by hematopoietic cells in a single sample. Our antibody arrays eliminate the time-consuming gel electrophoresis and protein transfer steps required for a Western blot. If you can collect data from a Western blot, you have the equipment to run an array experiment today.

### Features

- Determines the expression level of over 100 soluble receptors simultaneously
- Easier to perform than a Western blot
- No specialized equipment is required

## View Pathway



[rndsystems.com/pathways\\_hsc](https://rndsystems.com/pathways_hsc)

## Hematopoietic Stem Cells

Hematopoietic stem cells (HSCs) are multipotent, self-renewing progenitor cells from which all differentiated blood cell types arise during the process of hematopoiesis. These cells include lymphocytes, granulocytes, and macrophages of the immune system as well as circulating erythrocytes and platelets. Classically, HSCs are thought to differentiate into two lineage-restricted, lymphoid and myelo-erythroid, oligopotent progenitor cells. An alternative, “myeloid-based” model for blood lineage development from HSCs describes a novel intermediary, a common myelo-lymphoid progenitor cell, which has the capacity to generate progeny from both lineages. The mechanisms controlling HSC homing to the bone marrow, self-renewal, and differentiation are thought to be influenced by a diverse set of cytokines, chemokines, receptors, and intracellular signaling molecules. R&D Systems and Tocris Bioscience offer a wide range of tools to isolate/culture, verify, differentiate, and investigate HSCs.

R&D SYSTEMS

NOVUS  
BIOLOGICALS

TOCRIS

protein simple

biotechne®

Global [info@bio-techne.com](mailto:info@bio-techne.com) [bio-techne.com/find-us/distributors](http://bio-techne.com/find-us/distributors) TEL +1 612 379 2956  
North America TEL 800 343 7475 Europe | Middle East | Africa TEL +44 (0)1235 529449  
China [info.cn@bio-techne.com](mailto:info.cn@bio-techne.com) TEL +86 (21) 52380373

[bio-techne.com](http://bio-techne.com)

