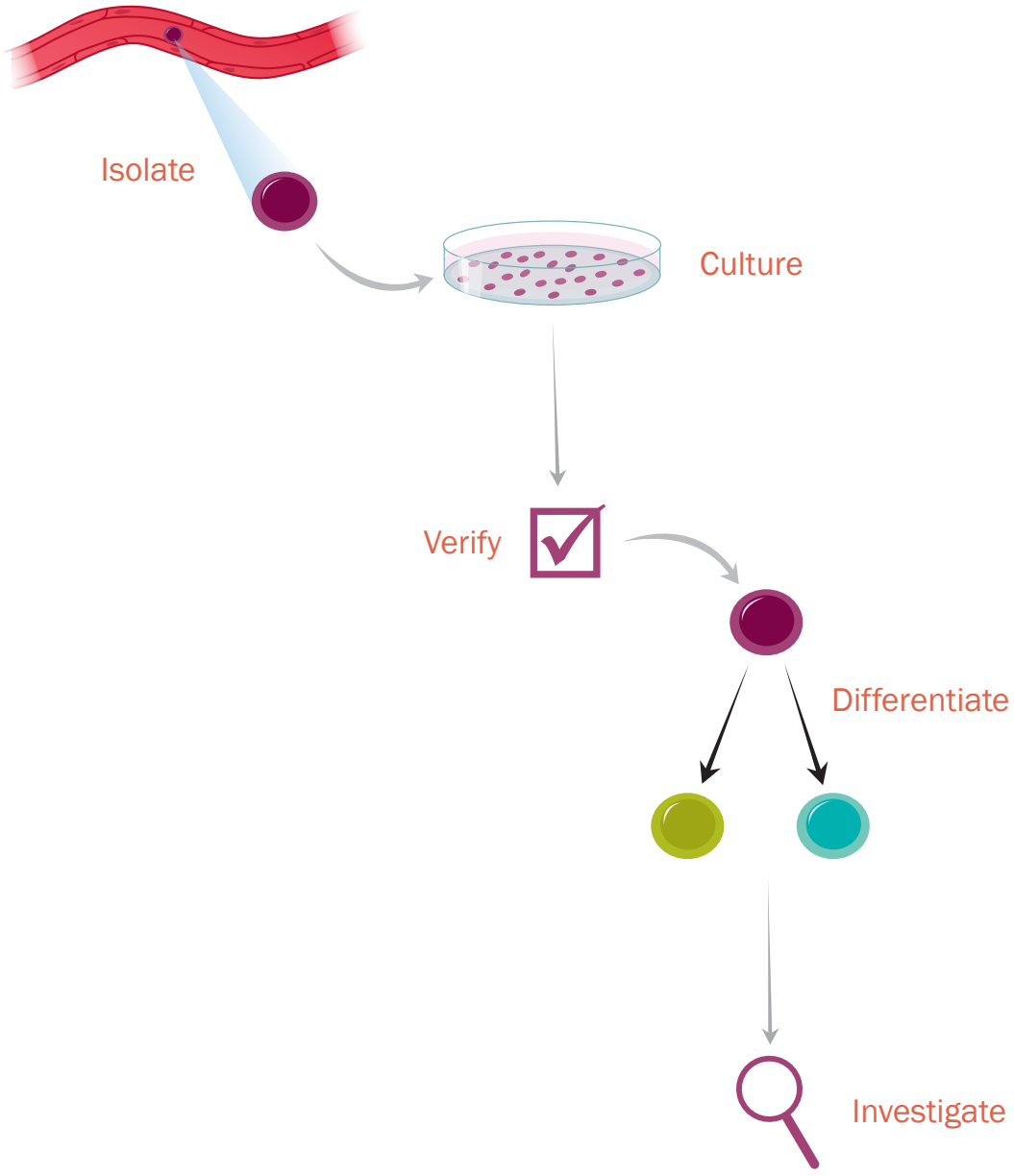
Hematopoietic Stem Cells



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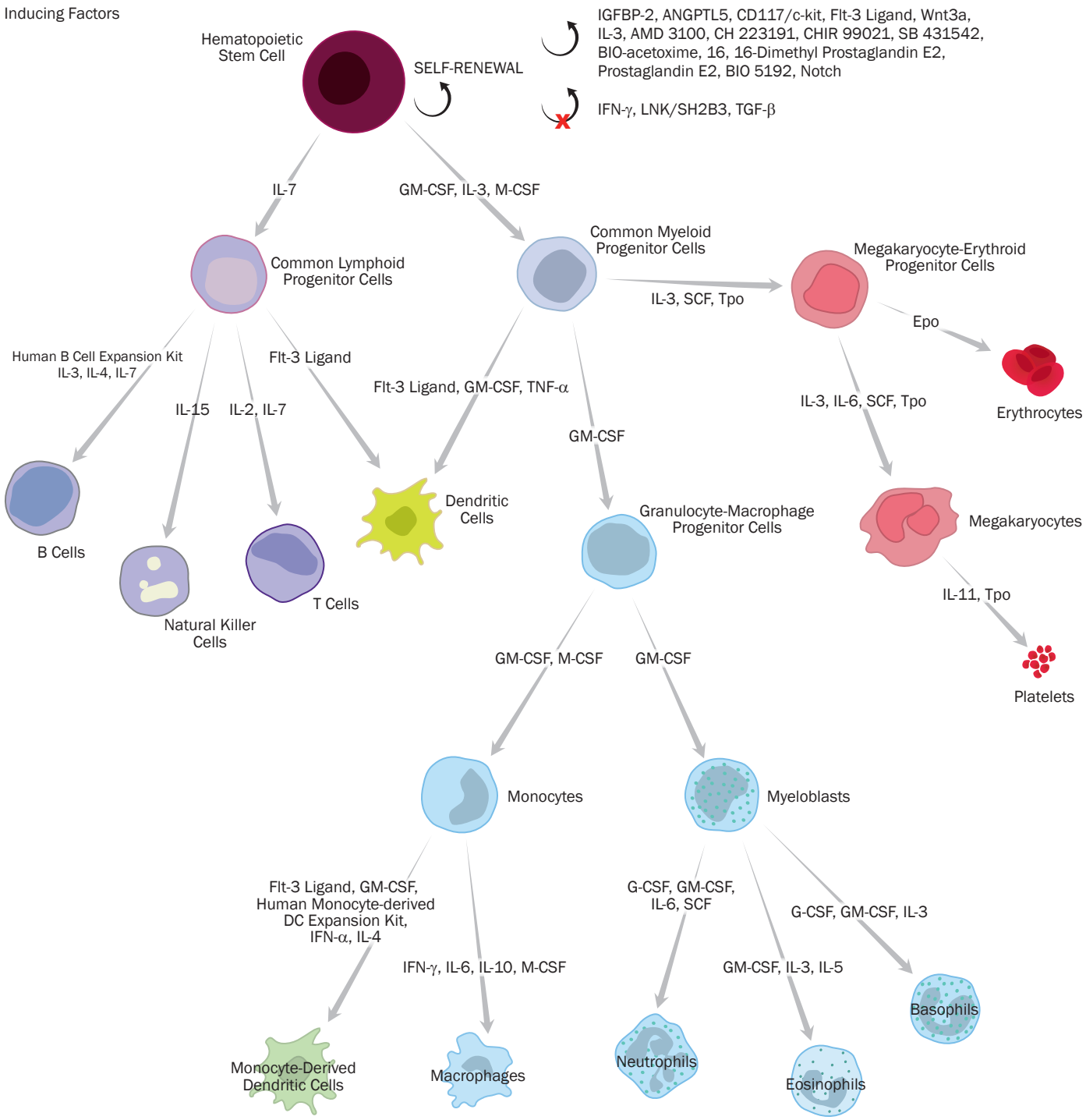
Hematopoietic Stem Cells

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.



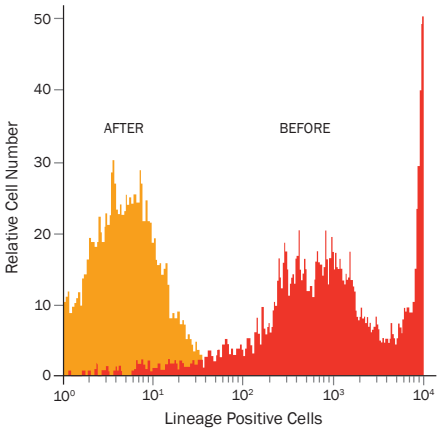
Isolate and Culture

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 MagCellect™ Mouse Hematopoietic Cell Lineage Depletion Kit. The MagCellect 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 R&D Systems MagCellect 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, MagCellect streptavidin ferrofluid, blocking buffer, and wash buffer
- **Robust** – each kit processes up to 1 x 10^9 cells



Depletion of Lineage-committed Hematopoietic Cells. Lineage marker reactivity on BALB/c bone marrow (BM) cells processed with the MagCellect 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 x 10^9 bone marrow-derived cells

Product	Description	Catalog #
Mouse B220/CD45R	Monoclonal Rat IgG_{2A} (Clone RA3-6B2)	MLDP7
Mouse CD3	Monoclonal Rat IgG_{2B} (Clone 17A2)	MLDP1
Mouse CD4	Monoclonal Rat IgG_{2B} (Clone GK1.5)	MLDP2
Mouse CD5	Monoclonal Rat IgG_{2A} (Clone 53-7.3)	MLDP3
Mouse CD8α	Monoclonal Rat IgG_{2A} (Clone 53-6.7)	MLDP4
Mouse Integrin αM/CD11b/MAC-1	Monoclonal Rat IgG_{2B} (Clone M1/70)	MLDP5
Mouse Gr-1/Ly-6G	Monoclonal Rat IgG_{2B} (Clone RB6-8C5)	MLDP6
Mouse TER-119 Erythroid Antigen	Monoclonal Rat IgG_{2B} (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.

Cytokines and Growth Factors - Mouse Hematopoietic Stem Cell Expansion Cytokine Panel (Catalog # SMPK9)

Features

- Contains 100 µg of Recombinant Mouse Flt-3 Ligand, Tpo, and SCF
- Optimized for efficient HSC expansion

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

Factors for HSC Expansion	Catalog #	
Molecule	**Human**	**Mouse**
Angiopoietin-like Protein 5/ANGPTL5	6675-AN	
CD117/c-kit	332-SR	1356-SR
Flt-3 Ligand	308-FK	427-FL
IGFBP2	674-B2	797-B2
IL-3	203-IL	403-ML
SCF/c-kit	255-SC	455-MC
Thrombopoietin /Tpo	288-TP	488-TO

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 #
Tocriscreen Stem Cell Toolbox	High-throughput and high content screening	5060
AMD 3100 octahydrochloride	Mobilization of HSCs; HSC expansion	3299
CH 223191	HSC expansion	3858
CHIR 99021	HSC expansion	4423
SB 431542	HSC expansion	1614
BIO-acetoxime	HSC expansion	3874
16, 16-Dimethyl Prostaglandin E2	HSC expansion	4027
Prostaglandin E2	HSC expansion and homing; increases HSC engraftment	2296
Shz 1	Induces differentiation in M-PBMCs	4923

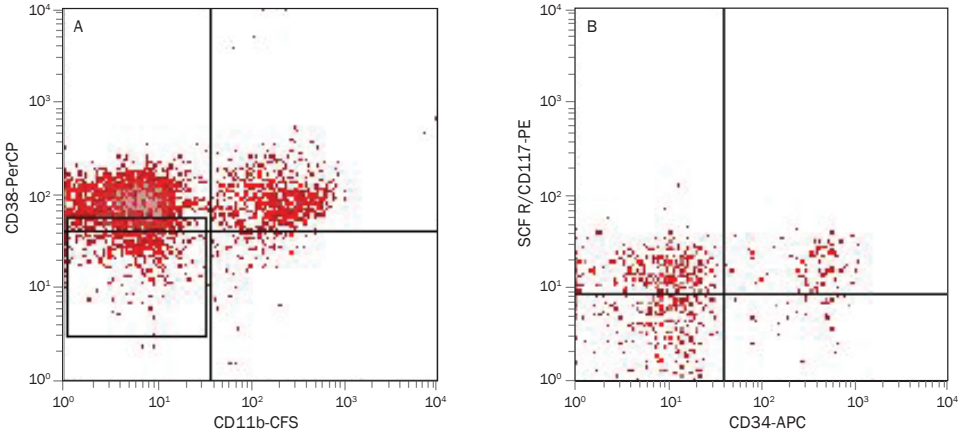
Verify

Hematopoietic Progenitor Cell Multi-Color Flow Cytometry Kits

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 Mouse and Human Hematopoietic Progenitor Cell Multi-Color Flow Cytometry Kits.

Features

- Verifies hematopoietic progenitor cell multipotency
- Defines the starting population to reduce experimental variation
- Simultaneously detects 3 mouse (Catalog # FMC005) or 4 human (Catalog # FMC019) established multipotency markers

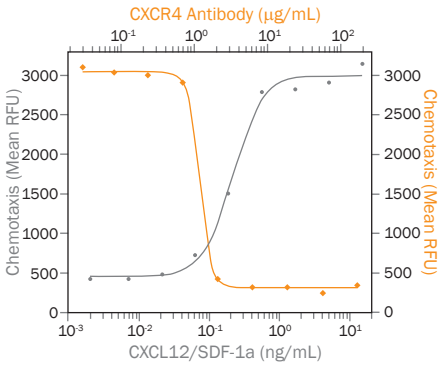


Verification of Human Umbilical Cord-derived Hematopoietic Progenitor Cell Identity using Multi-Color Flow Cytometry. Human umbilical cord blood cells were stained using reagents supplied in the Human Hematopoietic Progenitor Cell 4-Color Flow Kit (Catalog # FMC019). Cells negative for CD11b and negative/low 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.

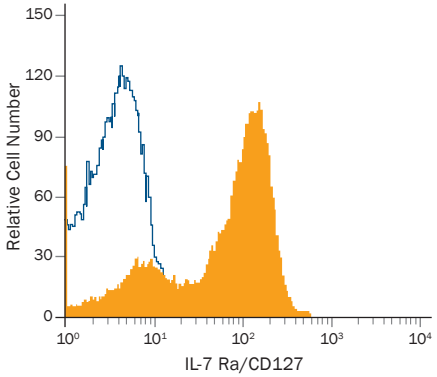
Individual Antibodies for HSC Characterization

Features

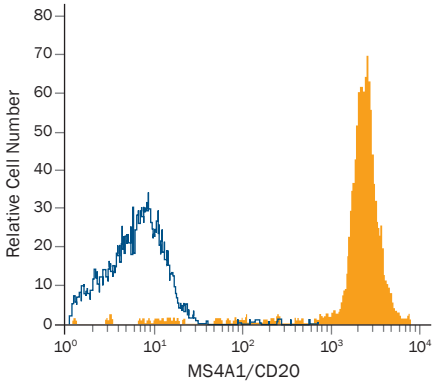
- **Reliable** – undergo rigorous quality testing to ensure lot-to-lot consistency and outstanding performance
- **Specific** – tested for cross-reactivity with related molecules by direct ELISA
- Neutralizing antibodies are tested to ensure low endotoxin levels
- Available in bulk pack sizes



Chemotaxis Induced by CXCL12/SDF-1α and Neutralized by a CXCR4 Antibody. Recombinant Human/Feline/Rhesus Macaque CXCL12/SDF-1α (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 # AR002). Chemotaxis elicited by 1 ng/mL Recombinant Human/Feline/Rhesus Macaque CXCL12/SDF-1α is neutralized (orange line) by increasing concentrations of a Mouse Anti-Human CXCR4 Monoclonal Antibody (Catalog # MAB173).



Detection of IL-7 Rα/CD127 by Flow Cytometry. Human peripheral blood lymphocytes were stained with an APC-conjugated Mouse Anti-Human IL-7 Rα/CD127 Monoclonal Antibody (Catalog # FAB306A; filled histogram) or an APC-conjugated Mouse IgG1 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 IgG1 Isotype Control (Catalog # IC002F; open histogram).

Differentiate

CellXVivo™ Differentiation and Expansion Kits

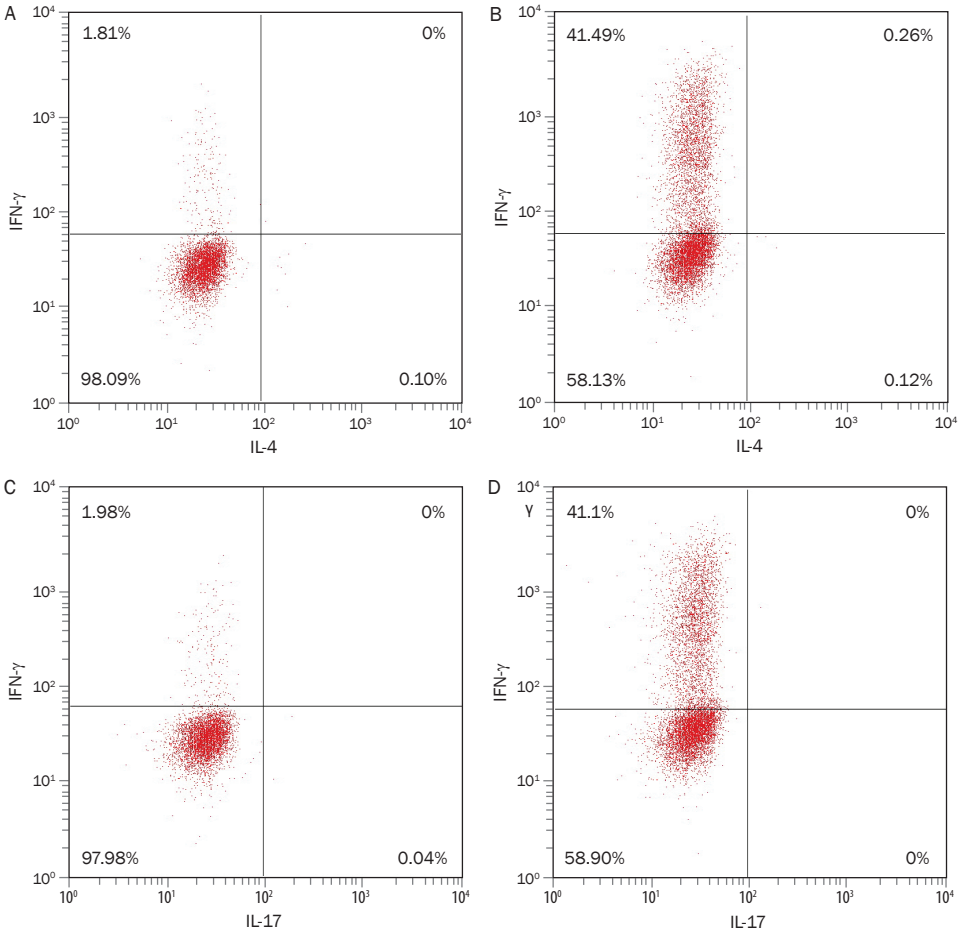
R&D Systems offers CellXVivo kits containing our high quality cytokines to differentiate and expand mature lymphoid and myeloid immune cells from enriched populations of human peripheral blood mononuclear cells (PBMCs). *Ex vivo* differentiation of leukocytes into immune effector cells limits the variability 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

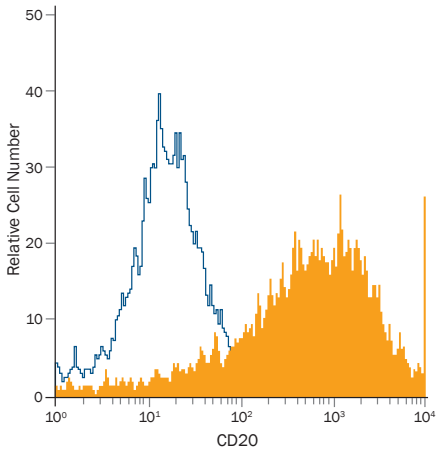
CellXVivo Kit	Catalog #
Human Th1 Cell Differentiation Kit	CDK001
Human Th2 Cell Differentiation Kit	CDK002
Human Th17 Cell Differentiation Kit	CDK003 Coming Soon
Human Monocyte-Derived Dendritic Cell Differentiation Kit	CDK004
Human B Cell Expansion Kit	CDK005

Th1 Differentiation



Verification of Th1 Cell Identity using Flow Cytometry. Human peripheral blood naïve CD4+ T cells without (A, C) and with (B, D) a 5 day differentiation using the reagents included in the CellXVivo Human Th1 Cell Differentiation Kit (Catalog # CDK001). (A, B) The cells were stained with an APC-conjugated Mouse Anti-Human IFN-γ Monoclonal Antibody (Catalog # IC285A) and a PE-conjugated Mouse Anti-Human IL-4 Monoclonal Antibody (Catalog # IC204P). (C, D) The cells were stained with an APC-conjugated Mouse Anti-Human IFN-γ Monoclonal Antibody (Catalog # IC285A) and a PerCP-conjugated Mouse Anti-Human IL-17 Monoclonal Antibody (Catalog # IC3171C). Control cultures were used to place the quadrants.

B Cell Expansion

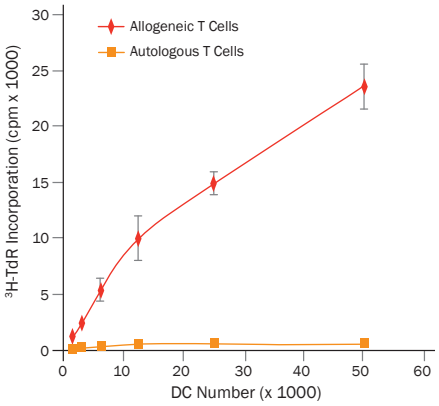


Detection of CD20 in Human B Cells. Human B cells were expanded for 5 days using reagents included in the CellXVivo Human B Cell Expansion Kit (Catalog # CDK005). The cells were labeled with a PE-conjugated Mouse Anti-Human CD20 Monoclonal Antibody (Catalog # FAB4225P; filled histogram) or a PE-conjugated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

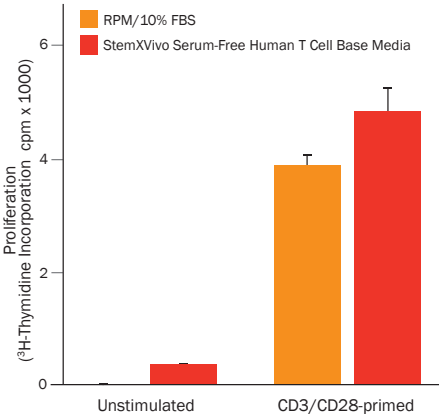


Mature Monocyte-derived Dendritic Cells Induce Proliferation of Allogenic T Cells. CD14$^+$ monocytes were cultured for seven days in Human StemXVivo Serum-free Dendritic Cell Base Media (Catalog # CCM003) supplemented with Recombinant Human GM-CSF (Catalog # 215-GM), Recombinant Human IL-4 (Catalog # 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 x 105 autologous or allogenic CD3$^+$ T cells for five days. 3H-thymidine (^{3}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 x 10^5 purified CD3$^+$ 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$^+$ T cells were cultured on 96 well microplates coated with Mouse Anti-Human CD3 ε (Clone UCHT1; Catalog # MAB100) and Goat Anti-Human CD28 Antigen Affinity-purified Polyclonal Antibody. [^{3}H]-thymidine was added for the final 18 hours. Cells were harvested and the incorporation of [^{3}H]-thymidine was measured using a beta-scintillation counter. Results are presented as the mean ± standard deviation of samples run in triplicate.

Investigate

Proteome Profiler™ Human Soluble Receptor Antibody 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

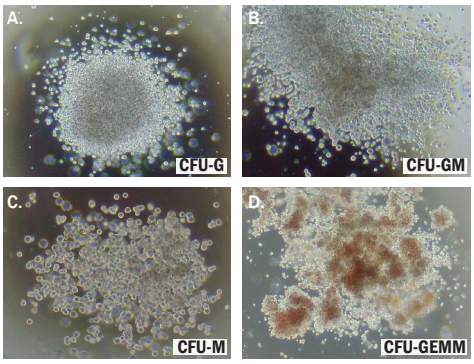
The Colony Forming Cell (CFC) Assay for HSC Characterization

The CFC assay is the standard *in vitro* assay for quantifying clonogenic progenitors from bone marrow, umbilical cord blood, and peripheral blood. The assay relies on the ability of hematopoietic progenitors to proliferate and differentiate into distinct colonies in a semi-solid media in response to cytokine stimulation.

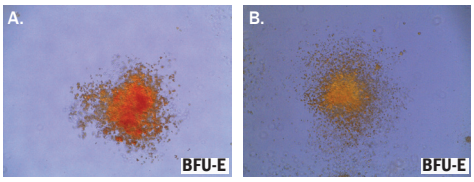
R&D Systems Methylcellulose-Based Reagents

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 optical clarity facilitates easier colony identification
- 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 Colony forming unit-macrophage (CFU-M) and CFU-G descriptions. **C.** CFU-M are clonogenic progenitors of macrophages that give rise to a ho-mogenous population of macrophages. **D.** Colony forming unit-granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM) are multi-lineage progenitors that give rise togranulocyte, 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**).

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