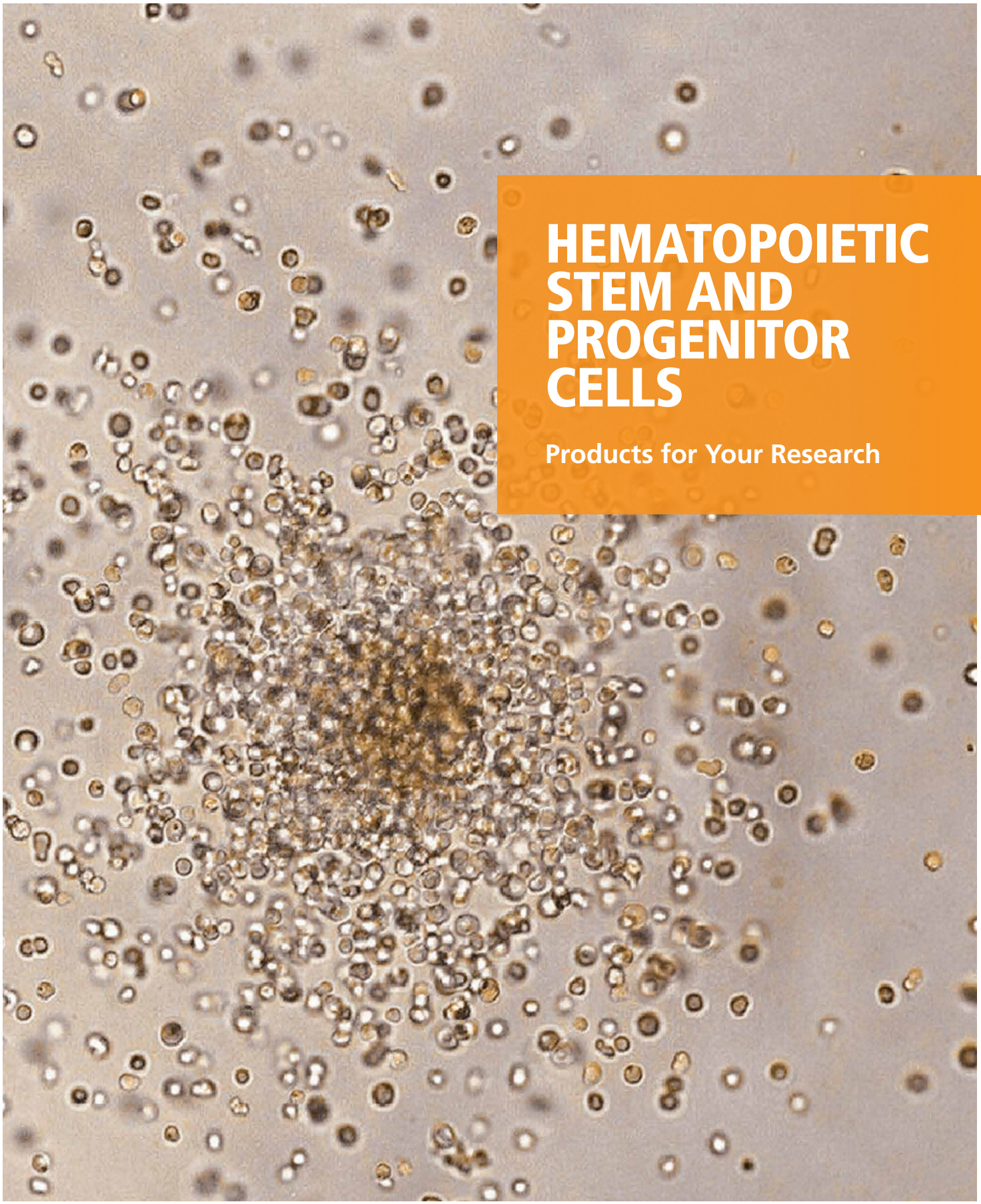

HEMATOPOIETIC STEM AND PROGENITOR CELLS

Products for Your Research

STEMCELL™ TECHNOLOGIES

Scientists Helping Scientists™ | WWW.STEMCELL.COM

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Your Ideas. Our Tools.

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

STEMCELL Technologies is the world leader in developing tools for hematology research. Our portfolio includes a comprehensive range of products for cell sourcing & isolation, expansion & differentiation, and analysis of hematopoietic stem and progenitor cells (HSPCs). By using STEMCELL products from start to finish, you will ensure standardization throughout your HSPC research.

STEMCELL Products for Every Step of Your HSPC Research

Cell Sourcing & Isolation	Expansion & Differentiation	Analysis
Primary Human Hematopoietic Cells	StemSpan™ Serum-Free Media	MyeloCult™ Media
Cell Isolation Products & Platforms	StemSpan™ Supplements	MethoCult™ Media
• EasySep™	Recombinant Cytokines	STEMvision™ Instrument
• RoboSep™	Small Molecules	MegaCult™ Media
• SepMate™		Antibodies and ELISA Kits
• RosetteSep™		ALDH^{br} Assay Kit
• ErythroClear™		Proficiency Testing Programs
• HetaSep™		Contract Assay Services
Cryopreservation Media		

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

Cell Sourcing & Isolation | Expansion & Differentiation | Analysis

Primary Human Hematopoietic Cells

It All Starts with the Right Cells

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

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

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

Why Use STEMCELL's Primary Cells?

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

ACCESSIBLE. Obtain large lots of rare cell populations including hematopoietic stem and progenitor cells.

CONVENIENT. Start experiments on your schedule without being restricted by the availability of tissue.

FLEXIBLE. Reserve large numbers of cryopreserved cells while you test in your specific applications.

EFFICIENT. Use isolated primary cells to reduce the time spent collecting and culturing primary cells.

Donor Criteria

All human primary cell products are ethically sourced using informed consent forms (ICFs) and protocols approved by either the Food and Drug Administration (FDA) or an Institutional Review Board (IRB), ensuring the protection of personal information and donor anonymity. All donations are performed in the United States in compliance with all applicable federal, state and local laws, regulations, and guidance. Donors are pre-screened for general health and viral status, including HIV-1, HIV-2, Hepatitis B, and Hepatitis C.**

Figure 1. Fresh Whole Bone Marrow

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

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

** Leuko Pak, Whole Blood, and Bone Marrow (LP, WB, and BM) Donor Screening: 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 negative test results. Fresh LP, WB, and BM: If the donor has tested negative within 90 days prior to donation, the product will be shipped with negative test results. 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). Cord Blood (CB) Donor Screening: Testing for HIV-1, HIV-2, Hepatitis B, and Hepatitis C is performed on a maternal blood sample and/or on a sample of the donated cord blood. Cryopreserved CB: Products are shipped with negative test results from the donor screening.

Product Warranty

STEMCELL Technologies warrants that primary cell products meet the claimed product specifications, including viable cell number and purity, when the recommended protocols are followed. STEMCELL Technologies assures its cells to be viable and cell numbers recovered to be accurate, when handled exactly according to our instructions for thawing and counting (available at www.stemcell.com). STEMCELL Technologies cannot guarantee biological function or any other properties associated with performance of cells in researchers' individual assay systems.

Fresh Human Bone Marrow Products*

DESCRIPTION	ANTICOAGULANT	QUANTITY	CATALOG #
Whole Bone Marrow	Heparin	> 25 mL	70502.2
		> 50 mL	70502.1
		> 100 mL	70502

Fresh Human Peripheral Blood Products*

DESCRIPTION	ANTICOAGULANT	QUANTITY	CATALOG #
Peripheral Blood Leuko Pak[1]	ACDA[2]	Quarter Size	70500.2
		Half Size	70500.1
		Full Size	70500

[1] A full size Leuko Pak typically contains 1.1 ± 0.3 x 10^{10} cells and has a volume of approximately 120 mL.

[2] ACDA: acid citrate dextrose solution A.

Cryopreserved Human Umbilical Cord Blood Products*

DESCRIPTION	QUANTITY	CATALOG #
Mononuclear Cells (MNCs)	15 x 10^6 cells	70007.1
	50 x 10^6 cells	70007.2
	150 x 10^6 cells	70007
CD34$^+$ Cells (Mixed Donor)	0.2 x 10^6 cells	70008.1
	0.5 x 10^6 cells	70008.3
	1 x 10^6 cells	70008
	5 x 10^6 cells	70008.6
CD34$^+$ Cells (Single Donor)	0.2 x 10^6 cells	70008.2
	0.5 x 10^6 cells	70008.4
	1 x 10^6 cells	70008.5

Cryopreserved Human Bone Marrow Products*

DESCRIPTION	QUANTITY	CATALOG #
Mononuclear Cells (MNCs)	5 x 10^6 cells	70001.1
	15 x 10^6 cells	70001.2
	25 x 10^6 cells	70001
	50 x 10^6 cells	70001.3
	100 x 10^6 cells	70001.4
CD34$^+$ Cells	0.1 x 10^6 cells	70002.1
	0.3 x 10^6 cells	70002.2
	0.5 x 10^6 cells	70002.3
	1 x 10^6 cells	70002
	2 x 10^6 cells	70002.4
	5 x 10^6 cells	70002.5

Cryopreserved Human Peripheral Blood Products*

DESCRIPTION	QUANTITY	CATALOG #
Mononuclear Cells (MNCs)	15 x 10^6 cells	70025.1
	25 x 10^6 cells	70025.2
	50 x 10^6 cells	70025.3
	100 x 10^6 cells	70025
CD34$^+$ Cells	0.2 x 10^6 cells	70040
	0.5 x 10^6 cells	70040.1
	1 x 10^6 cells	70040.2

Cryopreserved Mobilized Human Peripheral Blood Products*

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

For a complete listing of fresh and cryopreserved products, visit **www.stemcell.com/primarycells**.

* (see previous page)

Cell Isolation Products & Platforms

Fast and Easy Hematopoietic Stem and Progenitor Cell Isolation

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

EasySep™

Immunomagnetic Progenitor Cell Isolation

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

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

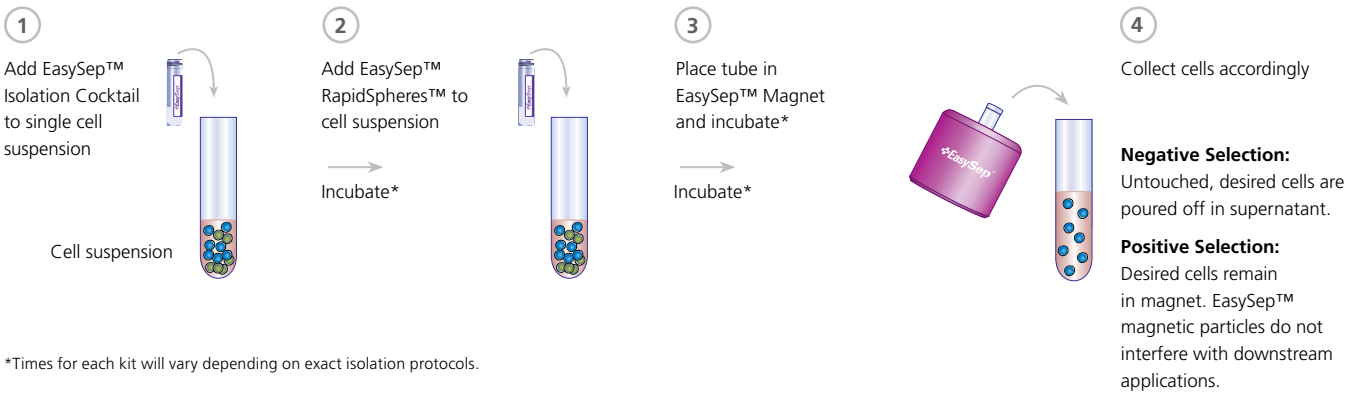
Why Use EasySep™ ?

FAST AND EASY. Isolate cells in as little as 8 minutes with a simple pour.

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

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

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



*Times for each kit will vary depending on exact isolation protocols.

Figure 2. Typical EasySep™ Human Cell Isolation Protocol

RoboSep™

RoboSep™ instruments offer true walk-away automation of immunomagnetic cell separation. Using EasySep™ reagents, RoboSep™-S and RoboSep™-16 perform all cell labeling and magnetic isolation steps for up to four and sixteen samples, respectively. Sample handling is minimized and the use of disposable tips in this column-free system eliminates cross-contamination while ensuring that isolated cells of interest are immediately available for any downstream application.


RoboSep™-S


RoboSep™-16

SepMate™

Hassle-Free PBMC Isolation

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

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

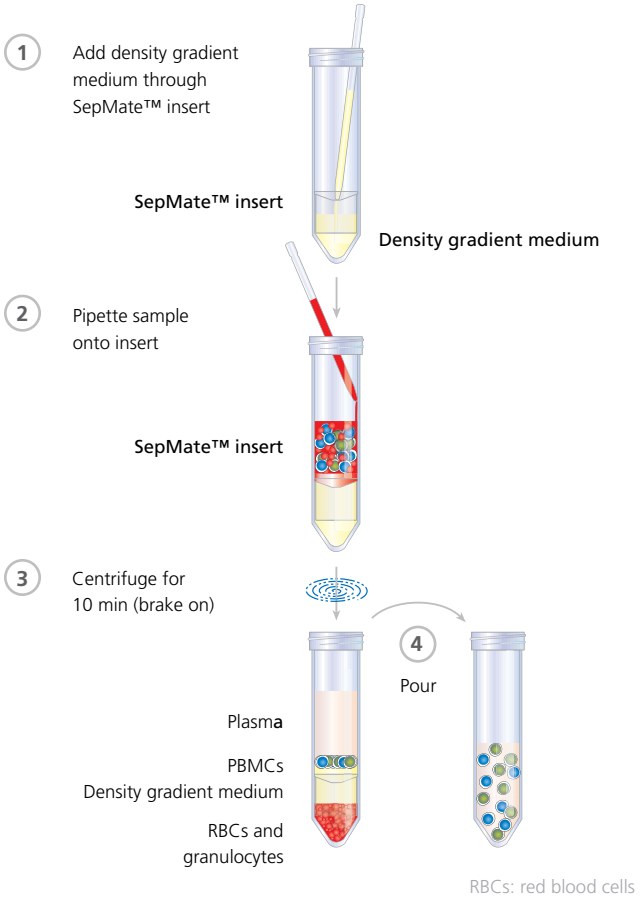


Figure 3. SepMate™ Procedure

Why Use SepMate™ ?

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

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

CONSISTENT. Eliminate errors and minimize variability between users.

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

Products

PRODUCT NAME	CATALOG #	BLOOD VOLUME PROCESSED	UNIT SIZE
SepMate™-15 (IVD[1])	85415	0.5 - 5 mL	100 tubes
SepMate™-15 (RUO[2])	86415		
SepMate™-50 (IVD[1])	85450	4 - 17 mL	
SepMate™-50 (RUO[2])	86450		

PRODUCT NAME	CATALOG #	DENSITY	UNIT SIZE
Lymphoprep™	07801 07851 07811 07861	1.077 g/mL[3]	250 mL 500 mL 4 x 250 mL 6 x 500 mL

[1] SepMate™ 85415/85450 is available in Australia, Canada, Europe, and the United States of America, where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells from human whole blood, cord blood, and bone marrow by density gradient centrifugation. This product is also available in China where it is considered a non-medical device by the China Food and Drug Administration (CFDA), and should therefore be used as general laboratory equipment.

[2] SepMate™ 86415/86450 is intended for Research Use Only (RUO) and is available in regions where SepMate™ 85415/85450 is not available.

[3] Lymphoprep™ has the same density as Ficoll-Paque® and can be substituted for Ficoll-Paque® without any need to change your existing protocols. Lymphoprep™ is for Research Use Only (RUO).

RosetteSep™

Unique Immunodensity Cell Isolation (Non-Magnetic)

RosetteSep™ is a fast and easy immunodensity procedure for the isolation of untouched cells directly from whole blood. By crosslinking unwanted cells to red blood cells present in the sample, immunorosettes are formed (Figure 4). These immunorosettes pellet during density gradient centrifugation (e.g. using Lymphoprep™), leaving untouched and highly purified target cells at the interface between the plasma and the density gradient medium. For example, RosetteSep™ can be used to enrich for $CD34^+$ cells by depleting lineage-committed cells from blood or bone marrow.

Combine RosetteSep™ and SepMate™ for Easy Isolation of Cell Subsets

Using the new SepMate™ tube makes using RosetteSep™ even faster and easier. The SepMate™ insert creates a physical barrier between the sample and density gradient medium, allowing the sample to be rapidly pipetted or poured into the tube. Centrifugation time is reduced to just 10 minutes, and purified target cells can simply be poured off into a new tube.

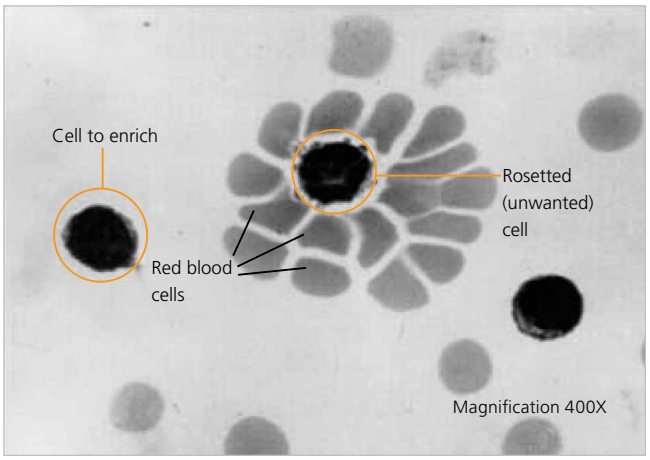


Figure 4. Image of a Blood Sample After Addition of the RosetteSep™ Cocktail and Prior to Density Gradient Centrifugation

Why Use RosetteSep™ ?

FAST AND EASY. Isolate untouched cell subsets from whole blood during your standard density gradient centrifugation step.

VERSATILE. Choose from a wide range of RosetteSep™ reagents to isolate the cell subset of your interest.

HIGH PURITY. Obtain highly purified, unlabeled and viable cells that are immediately ready for downstream analysis.

FLEXIBLE. Combine RosetteSep™ with SepMate™ to minimize variability between separations.

The RosetteSep™ and SepMate™ Procedure

Incubate the blood sample with RosetteSep™ at room temperature.

Add density gradient medium to SepMate™ tube, then rapidly pipet or pour the blood sample directly over the insert.

After centrifugation with the brake on, simply pour highly purified target cells into a new tube.

25 Minutes

Human CD34$^+$ Hematopoietic Progenitor Cell Isolation by Positive Selection

Applications

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

SOURCE	PRODUCT NAME	PURITY[1]	CAPACITY	CATALOG #
Mobilized PBMCs, CBMCs, BMMCs	EasySep™ Human CD34 Positive Selection Kit	84 - 99%	For labeling up to 5 x 10^9 cells	18056[2]
	RoboSep™ Human CD34 Positive Selection Kit			18056RF[2]
Whole Blood, Buffy Coat	EasySep™ Human Whole Blood CD34 Positive Selection Kit	26 - 41%	For labeling 75 mL whole blood (37 mL buffy coat)	18086
	RoboSep™ Human Whole Blood CD34 Positive Selection Kit			18086RF
Whole Blood	Complete Kit for Human Whole Blood CD34$^+$ Cells	79 - 95%	For labeling 120 mL whole blood	15086
	RoboSep™ Complete Kit for Human Whole Blood CD34$^+$ Cells			15086RF
Fresh Cord Blood	EasySep™ Human Cord Blood CD34 Positive Selection Kit II	91 ± 9%	For labeling 1000 mL cord blood	17896[3,4]
	RoboSep™ Human Cord Blood CD34 Positive Selection Kit II			17896RF[3,4]
	EasySep™ Human Cord Blood CD34 Positive Selection Kit III	87 ± 12%		17897[4]
	RoboSep™ Human Cord Blood CD34 Positive Selection Kit III			17897RF[4]
hESC and hiPSC Cultures	EasySep™ hESC-Derived CD34 Positive Selection Kit	84 - 99%	For labeling up to 5 x 10^9 cells	18167
PBMCs, Cord Blood, Bone Marrow	StemSep™ Human CD34 Positive Selection Cocktail	97 ± 2%	For labeling up to 2 x 10^9 cells	14756
			For labeling up to 1 x 10^{10} cells	14766

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

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

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

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

Human Hematopoietic Progenitor Cell Isolation by Negative Selection

Applications

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

SOURCE	PRODUCT NAME	PURITY[1]	CAPACITY	CATALOG #
Mobilized PBMCs, BMMCs	EasySep™ Human Progenitor Cell Enrichment Kit	42 ± 5 fold CD34$^+$ cell enrichment (bone marrow)	For labeling up to 1 x 10^9 cells	19056
	RoboSep™ Human Progenitor Cell Enrichment Kit			19056RF
PBMCs, CBMCs	EasySep™ Human Progenitor Enrichment Kit with Platelet Depletion	50 - 75%	For labeling up to 1 x 10^9 cells	19356[2]
	RoboSep™ Human Progenitor Enrichment Kit with Platelet Depletion			19356RF[2]
Bone Marrow	RosetteSep™ Human Bone Marrow Progenitor Cell Pre-Enrichment Cocktail	25 ± 10 fold CD34$^+$ cell enrichment	For labeling 40 mL bone marrow	15027
			For labeling 200 mL bone marrow	15067
Cord Blood	RosetteSep™ Human Hematopoietic Progenitor Cell Enrichment Cocktail	29 ± 9%	For labeling 40 mL cord blood	15026
			For labeling 200 mL cord blood	15066
	Complete RosetteSep™ Human Cord Blood Progenitor Enrichment Kit	29 ± 9%	For processing 500 mL cord blood	15276
PBMCs BMMCs CBMCs	StemSep™ Human Hematopoietic Progenitor Cell Enrichment Kit	74 - 88% 30 - 50% 45 - 61%	For labeling up to 1 x 10^9 cells	14056
			For labeling up to 5 x 10^9 cells	14066
Cord Blood	RosetteSep™ Human Cord Blood Debulking Cocktail	5 ± 1% (CD34$^+$ cells)	For labeling 40 mL cord blood	15126[3]
			For labeling 200 mL cord blood	15166[3]

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

Mouse Hematopoietic Progenitor Cell Isolation by Positive Selection

Applications

- Isolate mouse hematopoietic progenitors by positive selection.
- Select mouse hematopoietic progenitors using monoclonal antibodies to specific cell surface antigens associated with progenitor cell phenotypes (e.g. SCA1$^+$, c-KIT$^+$, AA4.1$^+$).

CELL TYPE	SOURCE	PRODUCT NAME	PURITY	CAPACITY	COMPATIBLE STAINING ANTIBODIES	CATALOG #
SCA1$^+$ Cells	Bone Marrow	EasySep™ Mouse SCA1 Positive Selection Kit	87 - 97%	For labeling up to 2 x 10^9 cells	60032 Anti-Mouse SCA1 Antibody	18756
		RoboSep™ Mouse SCA1 Positive Selection Kit				18756RF
CD117$^+$ (c-KIT) Cells	Bone Marrow	EasySep™ Mouse CD117 (c-KIT) Positive Selection Kit	88 - 95%	For labeling up to 2 x 10^9 cells	60025 Anti-Mouse CD117 (c-KIT) Antibody 60034 Anti-Mouse CD117 (c-KIT) Antibody	18757
		RoboSep™ Mouse CD117 (c-KIT) Positive Selection Kit				18757RF

Mouse Hematopoietic Progenitor Cell Isolation by Negative Selection

Applications

- Enrich mouse hematopoietic progenitors (including Lin$^-$SCA1$^+$c-KIT$^+$ or LSK cells) by depleting unwanted mature cell types using monoclonal antibodies targeted to specific cell surface antigens expressed on these cells (i.e. lineage depletion).
- Obtain unlabeled progenitor-enriched cell populations, i.e. lineage negative (Lin$^-$) cells.

CELL TYPE	SOURCE	PRODUCT NAME	PURITY	CAPACITY	COMPATIBLE STAINING ANTIBODIES	CATALOG #
Hematopoietic Progenitor Cells	Bone Marrow	EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Kit	60 - 84%	For labeling up to 1 x 10^9 cells	60001 Anti-Mouse CD11b Antibody 60006 Anti-Mouse CD19 Antibody 60015 Anti-Mouse CD3 Antibody 60019 Anti-Mouse CD45R Antibody 60028 Anti-Mouse Gr-1 Antibody 60033 Anti-Mouse TER119 Antibody	19856
		RoboSep™ Mouse Hematopoietic Progenitor Cell Isolation Kit				19856RF
		StemSep™ Mouse Hematopoietic Progenitor Cell Enrichment Kit	--	For labeling up to 1 x 10^9 cells		13056

For a full listing of cell isolation tools and reagents for mouse cells, visit **www.stemcell.com/HSPCworkflow** and select "Filter by: Mouse" under the "Cell Sourcing & Isolation" tab.

ErythroClear™

Depletion of Red Blood Cells from Small Volume Cord Blood Samples

ErythroClear™ Red Blood Cell Depletion Reagent and Magnet

Why Use ErythroClear™ ?

FAST. Process up to 16 samples at once in just a few minutes.

FLEXIBLE. Suitable for fresh or frozen cord blood.

SMALL VOLUMES. Optimized for small (50 - 100 µL) samples.

EFFICIENT. 99% of GlyAB$^+$ RBCs are depleted, without altering progenitor frequency.

PRODUCT NAME	CATALOG #	COMPONENTS
ErythroClear™ Red Blood Cell Depletion Kit	01739	1 magnet and 2 x 2 mL reagent*
	01738	2 x 2 mL reagent*

*Each kit is suitable for processing 2 mL of cord blood as 20 x 100 µL samples with an ErythroClear™ magnet.

Cord blood sample before (left) and after (right) depletion of RBCs with ErythroClear™

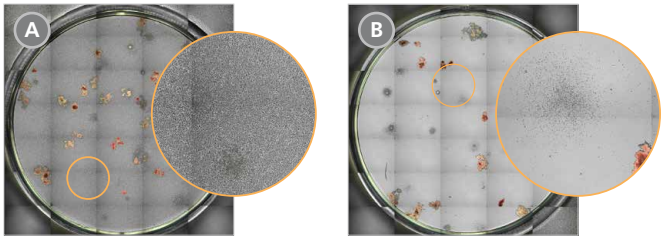


Figure 5. Improved Visualization of Cord Blood-Derived Colonies in CFU Assays (A) Without and (B) With RBC Depletion

The presence of large numbers of red blood cells (RBCs) in cord blood samples can reduce the accuracy of assays used to measure hematopoietic stem and progenitor cells (HSPCs), such as the colony-forming unit (CFU) assay, and flow cytometry assays for CD34 expression or aldehyde dehydrogenase (ALDH) activity. These assays are commonly used by cord blood banks to evaluate the quality of a cord blood unit prior to banking, as part of quality control of cryopreservation protocols, or to aid in selection of units for transplantation.

Typical methods for depleting RBCs often include long incubation or centrifugation steps, may not work well with frozen samples, or are not easily performed on small volume samples such as the segments attached to the main cord blood bag.

As part of our complete set of tools for standardization of the CFU assay, STEMCELL Technologies has developed the ErythroClear™ Red Blood Cell Depletion Kit. This kit enables the depletion of RBCs from up to 16 small (50 - 100 µL) cord blood samples at a time, in just a few minutes. The ErythroClear™ reagent contains immunomagnetic particles that bind to Glycophorin A/B expressing (GlyAB$^+$) cells, which are then selectively depleted using the ErythroClear™ magnet.

For a full listing of products for cord blood banking, visit **www.stemcell.com/CBworkflow**.

HetaSep™

HetaSep™

PRODUCT NAME	CATALOG #	SIZE
HetaSep™	07806	20 mL
	07906	100 mL

The presence of large numbers of RBCs in a colony-forming unit (CFU) assay prevents hematopoietic colonies from being accurately visualized either manually or using STEMvision™ (Figure 6). RBCs must be removed from fresh cord blood, bone marrow, and mobilized peripheral blood samples (whether whole or processed), before performing the CFU assay.

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

The HetaSep™ procedure does not affect the number of progenitor cells; 97% of CFUs are recovered in the RBC-cleared sample (Figure 7). HetaSep™-mediated RBC depletion requires only 50 µL of sample and is quick, making it easy to incorporate into an institution's workflow.

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

Why Use HetaSep™ ?

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

CONSISTENT. Recover > 97% of colonies.

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

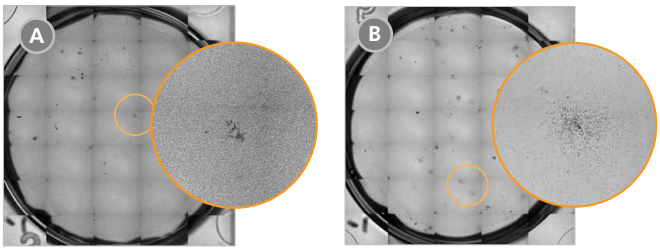


Figure 6. STEMvision™ Images of 7-Day CFU Assays of Fresh Cord Blood Samples Plated in MethoCult™ Express Without and With Prior Removal of RBCs Using HetaSep™

(A) Unacceptable background for a CFU assay. Note that fewer colonies are visible due to increased RBCs in culture. (B) Acceptable background (minimal RBCs) for a CFU assay.

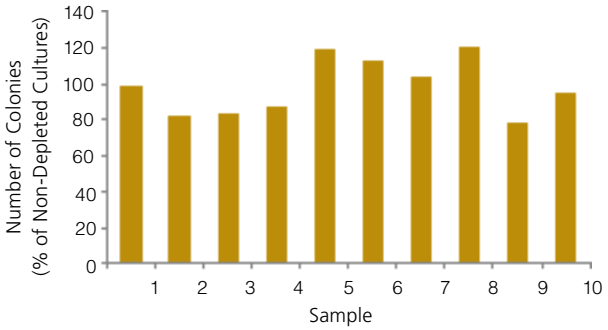


Figure 7. An Average of 97% of Colony-Forming Cells are Recovered Following RBC Depletion with HetaSep™

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

Cryopreservation Media

cGMP-Manufactured Freezing and Preservation Media Formulated with USP Grade Components

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

CryoStor® Freezing Media

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

BloodStor® Freezing Media

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

HypoThermosol® FRS Preservation Media

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

Product Information

PRODUCT NAME	CATALOG #	UNIT SIZE
CryoStor® CS10	07930	100 mL
	07955	100 mL bag
	07940	1000 mL bag
	07931	5 x 16 mL vials
	07959	5 x 10 mL vials
	07952	6 x 10 mL vials
CryoStor® CS5	07933	100 mL
	07953	100 mL bag
	07949	5 x 10 mL vials
CryoStor® CS2	07932	100 mL
HypoThermosol® FRS	07935	100 mL
	07936	500 mL
	07945	500 mL bag
	07934	16 x 10 mL vials
BloodStor® 55-5	07950	1000 mL bag
	07937	16 x 7.2 mL vials
BloodStor® 100	07951	50 mL
	07939	100 mL
	07938	5 x 100 mL

StemSpan™ Expansion Media & Supplements

Expansion and Differentiation of Hematopoietic Stem and Progenitor Cells

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

Applications

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

Serum-Free Expansion Media

MEDIUM	PRODUCT NAME	CATALOG # (SIZE)	RECOMMENDED FOR	COMPONENTS
Serum-Free	StemSpan™ SFEM	09600 (100 mL) 09650 (500 mL)	Culture of human HSPCs Culture of mouse, rat, and non-human primate HSPCs	Pre-tested BSA, insulin, transferrin, and supplements in IMDM
	StemSpan™ SFEM II	09605 (100 mL) 09655 (500 mL)	Expansion of human HSPCs Production of mature blood cells by expansion and lineage-specific differentiation of human HSPCs	
Animal Component-Free	StemSpan™-ACF	09855 (500 mL)	Culture of human HSPCs in the absence of human- or animal-derived components	Recombinant and synthetic components only
Xeno-Free	StemSpan™ H3000	09850 (500 mL)	Culture of human HSPCs in the absence of animal-derived components*	Human-derived or recombinant human proteins in IMDM

BSA: bovine serum albumin, IMDM: iscove's modified dulbecco's medium.
*Contains pre-tested human-derived and recombinant human proteins

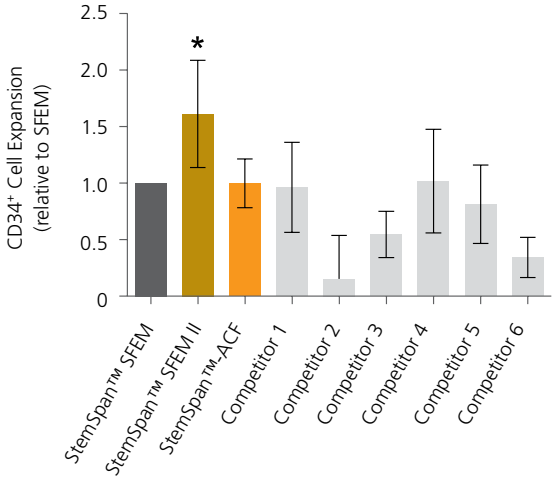


Figure 8. StemSpan™ SFEM II Serum-Free Expansion Medium Containing CD34+ Expansion Supplement Supports Greater Expansion of Human CD34+ Cells than Other Media Tested

Expansion of CD34+ cells, normalized relative to the values obtained in SFEM medium (dark gray bars) after culturing purified CD34+ CB cells for 7 days in StemSpan™ SFEM, SFEM II (gold bars), and -ACF (orange bars), and six media from other suppliers (light gray bars; Competitor 1 - 6, which included, in random order, X-VIVO™ 15 (Lonza), HP01 (Macopharma), StemPro®-34 (Life Technologies), CellGro® SCGM (Cellgenix), Stemline II™ (Sigma), and HPGM™ (Lonza). All media were supplemented with the StemSpan™ CD34+ Expansion Supplement. Vertical lines indicate 95% confidence limits. The numbers of cells produced in StemSpan™ SFEM II were significantly higher than in all other media (*$p < 0.01$, paired t-test, $n = 6$).

CD34+ Cell Expansion Supplements

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

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

Expansion and Lineage-Specific Differentiation Supplements and Kits

CELL TYPE	PRODUCT NAME	CATALOG # (SIZE)	RECOMMENDED FOR	COMPONENTS
Erythroid Progenitor Cells	StemSpan™ Erythroid Expansion Supplement	02692 (1 mL, 100X)	Stimulates the production of human erythroid cells by expansion and lineage-specific differentiation of human HPCs	rhSCF, rhIL-3, rhEPO
Megakaryocytes	StemSpan™ Megakaryocyte Expansion Supplement	02696 (1 mL, 100X)	Stimulates the production of human megakaryocytes by expansion and lineage-specific differentiation of human HPCs	rhSCF, rhTPO, rhIL-6, rhIL-9
Granulocytes	StemSpan™ Myeloid Expansion Supplement	02693 (1 mL, 100X)	Stimulates the production of human granulocytes by expansion and lineage-specific differentiation of human HPCs	rhSCF, rhTPO, rhG-CSF, rhGM-CSF
Monocytes	StemSpan™ Myeloid Expansion Supplement II	02694 (1 mL, 100X)	Stimulates the production of human monocytes by expansion and lineage-specific differentiation of human HPCs	rhFlt3L, rhSCF, rhTPO, rhM-CSF, rhGM-CSF and supplements
T Cells	StemSpan™ T Cell Generation Kit	09940 (Kit, various)	Stimulates the production of human T cells by expansion and lineage-specific differentiation of human HSPCs in stroma-free conditions	• SFEM II • Lymphoid Progenitor Expansion Supplement (10X) • Lymphoid Differentiation Coating Material (100X) • T Cell Progenitor Maturation Supplement (10X)
Myeloid Leukemic Cells	StemSpan™ Leukemic Cell Culture Kit	09720 (Kit, various)	Stimulates the production of human myeloid leukemia cells, allowing users to expand, culture, and use malignant cells for drug screening	• SFEM II • CD34+ Expansion Supplement • UM1171

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

Expansion of Human CD34+ Cells in Culture

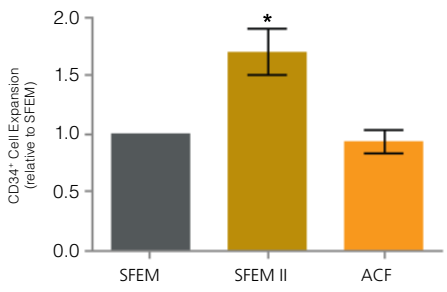


Figure 9. Expansion of Human Cord Blood-Derived CD34+ Cells Cultured in StemSpan™ Media Containing CD34+ Expansion Supplement

Purified human CB-derived CD34+ cells were suspended at a concentration of 10,000 cells per mL in StemSpan™ SFEM (gray bars), SFEM II (gold bars), and -ACF (orange bars) media containing CD34+ Expansion Supplement (Catalog #02691). Cultures were maintained for 7 days, after which time the cells were counted and examined for CD34 expression by flow cytometry. Shown are the fold expansion of CD34+ cells per input CD34+ cell (n = 6). Vertical lines indicate 95% confidence limits. The numbers of cells produced in StemSpan™ SFEM II were significantly higher than in SFEM (*$p < 0.001$; paired t-test, n = 6).

Differentiation of Human Progenitor Cells in Culture

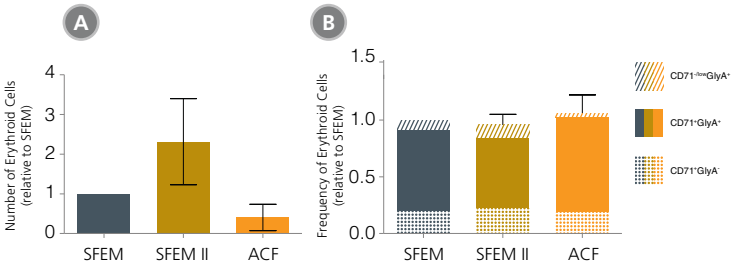


Figure 10. Expansion and Differentiation of Human Cord Blood-Derived CD34⁺ Cells Cultured in Different StemSpan™ Media Containing Erythroid Expansion Supplement

(A) Average numbers and (B) frequencies of erythroid cells normalized relative to the values obtained in StemSpan™ SFEM (grey bars) after culturing purified CD34⁺ CB cells (n = 6) for 14 days in StemSpan™ SFEM (gray bars), SFEM II (gold bars), or ACF (orange bars) media containing Erythroid Expansion Supplement. Vertical lines indicate 95% confidence limits. Erythroid cell yields were significantly higher in SFEM II compared to ACF ($p < 0.01$). Erythroid cell yields in SFEM II were also significantly higher than in SFEM for 5 of 6 CB samples tested ($p < 0.05$).

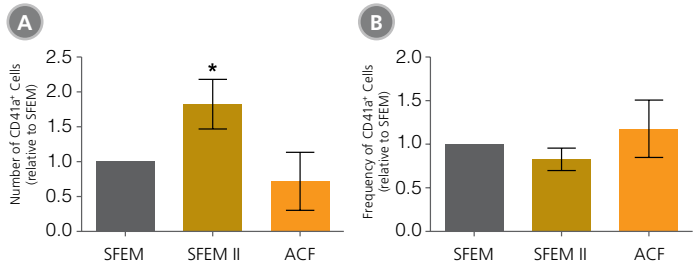


Figure 11. Expansion and Differentiation of Human Cord Blood-Derived CD34⁺ Cells Cultured in Different StemSpan™ Media Containing Megakaryocyte Expansion Supplement

(A) Average numbers and (B) frequencies of CD41⁺ megakaryocytes normalized relative to the values obtained in StemSpan™ SFEM (gray bars) after culturing purified CD34⁺ CB cells (n = 6) for 14 days in StemSpan™ SFEM, SFEM II (gold bars), and -ACF (orange bars) media containing Megakaryocyte Expansion Supplement. Vertical lines indicate 95% confidence limits. The numbers of CD41a⁺ cells were significantly higher in SFEM II compared to SFEM and -ACF medium (*$p < 0.01$; paired t-test, n = 6).

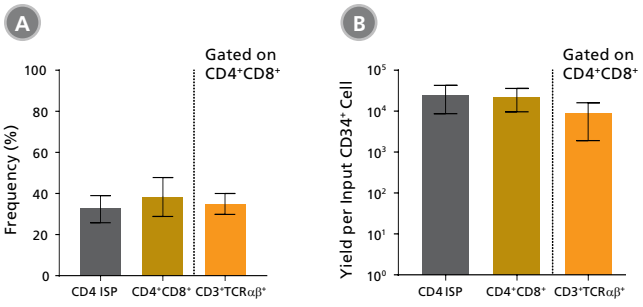


Figure 12. Frequency and Yield of CD4 Immature Single-Positive and CD4⁺CD8⁺ Double-Positive Cells After 42 Days of Culture

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

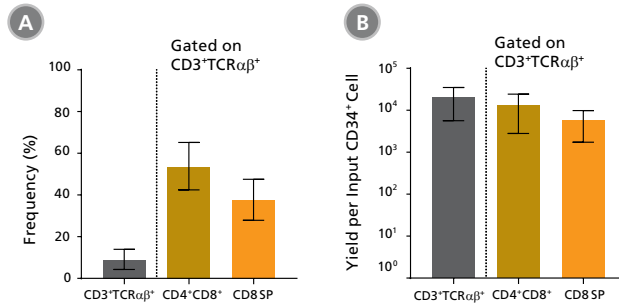


Figure 13. Frequency and Yield of CD8 Single-Positive T Cells After 49 Days of Culture

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

Table 1. Production of Myeloid Cells from Human Cord Blood-Derived CD34⁺ Cells Cultured in StemSpan™ SFEM II Containing Myeloid Expansion Supplement or Myeloid Expansion Supplement II

PRODUCT	TNCs PRODUCED PER INPUT CD34⁺ CELL*	MYELOID CELLS		
		% CD13⁺	% CD14⁺	% CD15⁺
StemSpan™ Myeloid Expansion Supplement (100X) (Catalog #02693)	5847 (2691 - 9003)	92 (89 - 95)	5 (3 - 8)	47 (39 - 55)
StemSpan™ Myeloid Expansion Supplement II (100X) (Catalog #02694)	2099 (933 - 3264)	94 (90 - 97)	74 (68 - 80)	11 (7 - 15)

Shown are numbers of total nucleated cells (TNCs) produced per input human CB-derived CD34⁺ cell and percentages of cells positive for myeloid markers CD13, CD14, and CD15 after 14 days of culture in StemSpan™ SFEM II containing Myeloid Expansion Supplement (n = 14) or Myeloid Expansion Supplement II (n = 16).
* Mean (95% confidence limits; the range within which 95% of results typically fall).

Recombinant Cytokines

Recombinant Human Cytokines

CYTOKINE	CATALOG #	UNIT SIZE
bFGF	78003	50 µg
BMP-2	78004	50 µg
BMP-4	02524	10 µg
EGF	78006	500 µg
EPO	78007	500 µg
FGF-4	78103	5 µg
FGF-7 (KGF)	78046	10 µg
FGF-8B	78008	10 µg
FGF-10 (KGF-2)	78037	10 µg
FGF-18	78041	10 µg
Flt3/Flk-2 Ligand	78009	100 µg
G-CSF	78012	100 µg
GM-CSF	78015	100 µg
IFN-γ	78020	100 µg
IGF-I	78022	500 µg
IGF-II	78023	50 µg
IL-1ß	78034	100 µg
IL-2	78036	50 µg
IL-3	78040	100 µg
IL-4	78045	100 µg
IL-5	78048	100 µg
IL-6	78050	100 µg
IL-6Rα	78083	10 µg
IL-7	78053	100 µg
IL-10	78024	50 µg
IL-11	78025	100 µg
IL-12	78027	25 µg
IL-15	78031	100 µg
IL-21	78082	10 µg
M-CSF	78057	100 µg
MIP-1α (CCL3)	78088	10 µg
MIP-1β (CCL4)	78090	5 µg
Oncostatin M	78094	10 µg
PDGF-AB	78096	10 µg
PDGF-BB	78097	10 µg
SCF	78062	100 µg
TGF-ß1	78067	5 µg
TNF-α	78068	50 µg
TPO	78070	100 µg
VEGF-165	78073	50 µg

Recombinant Mouse Cytokines

CYTOKINE	CATALOG #	UNIT SIZE
EGF	78016	10 µg
Flt3/Flk-2 Ligand	78011	100 µg
G-CSF	78014	100 µg
GM-CSF	78017	100 µg
IFN-γ	78021	100 µg
IL-1ß	78035	50 µg
IL-2	78081	20 µg
IL-3	78042	100 µg
IL-4	78047	100 µg
IL-5	78049	100 µg
IL-6	78052	100 µg
IL-7	78054	50 µg
IL-10	78079	10 µg
IL-11	78026	100 µg
IL-12	78028	100 µg
IL-13	78030	100 µg
IL-15	78080	10 µg
M-CSF	78059	100 µg
MIP-1α (CCL3)	78089	10 µg
MIP-1β (CCL4)	78091	10 µg
SCF	78064	100 µg
SDF-1α (CXCL12)	78121	5 µg
TNF-α	78069	100 µg
TPO	78072	50 µg
VEGF-164	78102	20 µg

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

Small Molecules for Human Hematopoietic Stem and Progenitor Cell Research

UM171, UM729, and StemRegenin 1

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

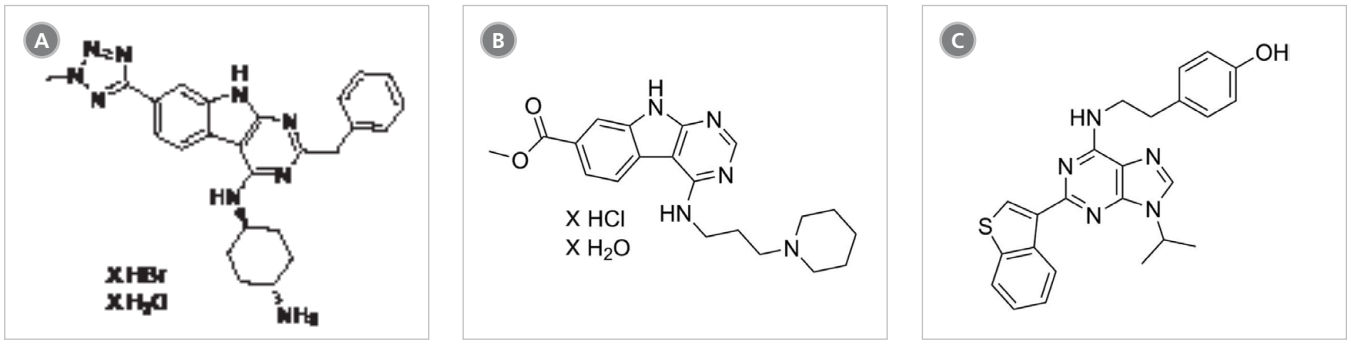


Figure 14. Chemical Structure of UM171, UM729 and StemRegenin 1

(A) UM171: (1r,4r)-N1-(2-benzyl-7-(2-methyl-2H-tetrazol-5-yl)-9H-pyrimido[4,5-b]indol-4-yl)cyclohexane-1,4-diamine
(B) UM729: Methyl 4-((3-(piperidin-1-yl)propyl)amino)-9H-pyrimido[4,5-b] indole-7-carboxylate
(C) StemRegenin 1 (SR1): 4-[2-[[2-benzo[b]thien-3-yl-9-(1-methylethyl)-9H-purin-6-yl]amino]ethyl]-phenol

Product Information

PRODUCT NAME	CATALOG #	SIZE	PATHWAY / TARGET	APPLICATIONS
UM171	72912 72914	250 µg 1 mg	Pyrimido-indole derivative that enhances HSC self-renewal in vitro	Expansion of human HSCs in culture
UM729	72332 72334	250 µg 1 mg		• Expansion of human HSCs in culture • Maintenance of LSCs in combination with SR1
StemRegenin 1	72342 72344	1 mg 5 mg	Aryl hydrocarbon receptor (AhR) antagonist	• Maintenance and expansion of human HSPCs in culture • Differentiation of human $CD34^+$ HPCs into functional dendritic cells • Maintenance of LSC activity in culture when combined with UM729
StemRegenin 1 (Hydrochloride)	72352 72354	1 mg 5 mg		

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

MyeloCult™ Long-Term Culture Media

Detection of Primitive Progenitor Cells

Long-Term Culture Media

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

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

Long-Term Culture-Initiating Cell Assay

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

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

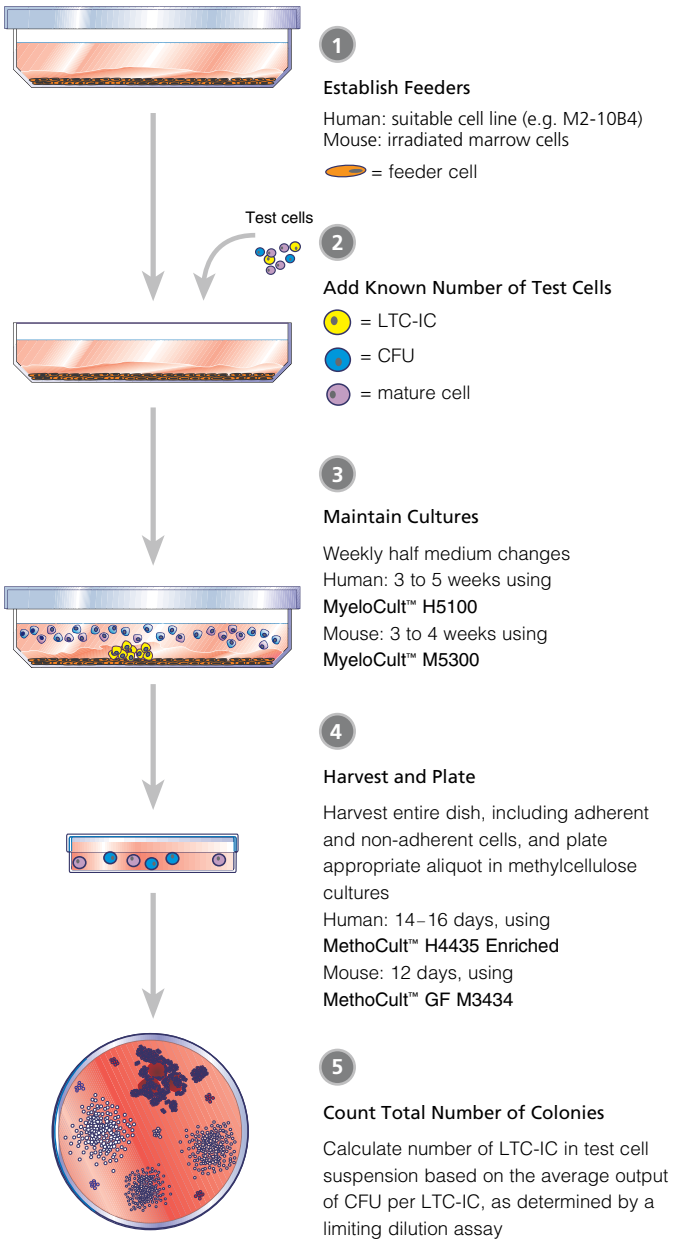


Figure 15. Bulk Culture LTC-IC Assay Procedure

Published Applications

Quantitate Frequency of LTC-IC and Study Their Phenotypic and Functional Properties. Primitive hematopoietic progenitors capable of initiating and sustaining myelopoiesis for several weeks in long-term culture have been called long-term culture-initiating cells (LTC-IC). The frequency of LTC-IC in human or mouse samples can be determined using the LTC-IC assay.[18-19,23] Experiments using MyeloCult™ and related products show that LTC-IC are a heterogeneous population of cells which can differ in phenotype, cell cycling characteristics, and expansion potential.[24-29]

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

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

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

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

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

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

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

PRODUCT NAME	CATALOG #	SIZE	APPLICATIONS
MyeloCult™ H5100	05100 05150	100 mL 500 mL	Myeloid long-term cultures of human hematopoietic progenitor cells and stromal feeder layers
MyeloCult™ M5300	05300 05350	100 mL 500 mL	Myeloid long-term cultures of mouse hematopoietic progenitor cells and stromal feeder layers

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

PRODUCT NAME	CATALOG #	SIZE	APPLICATIONS
MethoCult™ H4435 Enriched	04435 04445	100 mL 24 x 3 mL	Detection of human colonies derived from LTC-IC
MethoCult™ GF M3434	03434 03444	100 mL 24 x 3 mL	Detection of mouse colonies derived from LTC-IC

Support Products

PRODUCT NAME	CATALOG #	SIZE	APPLICATIONS
Hydrocortisone	07904	100 mg	Supplementation of MyeloCult™ media to a final concentration of 10^{-6} M Suitable for supplementing both human (Catalog #05150) and mouse (Catalog #05350) MyeloCult™ media for LTC-IC assays
M2-10B4 LTC-IC Assay Feeder Cell Line, Irradiated	00370	3 x 10^6 cells	Stromal cell line to support human myelopoiesis in long-term culture
M2-10B4 (IL-3, G-CSF) + sl/sl (IL-3, SCF) LTC-IC Assay Mixed Feeder Cell Lines, Irradiated	00371	3 x 10^6 cells	Mixed cell lines genetically engineered to produce IL-3 and SCF to support human myelopoiesis in the LTC-IC assay
M2-10B4 (IL-3, G-CSF) + sl/sl (IL-3, SCF) + sl/sl (FL) LTC-IC Assay Mixed Feeder Cell Lines, Irradiated	00372	3 x 10^6 cells	Mixed cell lines genetically engineered to produce IL-3, SCF, and Flt-3 ligand to support human myelopoiesis in the LTC-IC assay
L-Calc™ software	28600	N/A	Limiting dilution analyses for determination of LTC-IC frequencies and other applications NOTE: Free download of L-Calc™ Software is available at **www.stemcell.com**

MethoCult™ Media

for Performing Colony-Forming Unit Assays

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

Key features of the CFU assay:

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

Why Use MethoCult™ ?

STANDARDIZED. Prepared using carefully screened components.

CONSISTENT. Rigorous performance testing, ensuring superior lot-to-lot reproducibility.

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

FLEXIBLE. Also available in formulations that allow the researcher to add components of choice. Custom formulations and sizes are available on request.

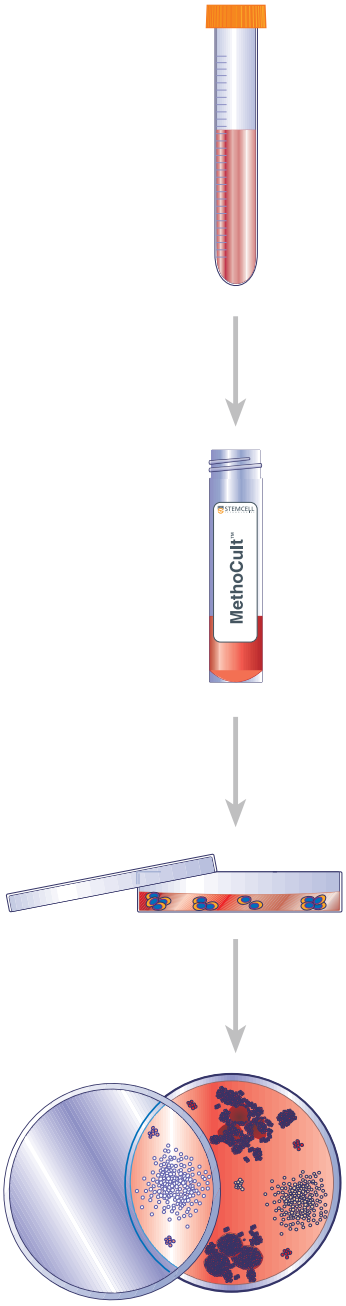
IVD. Formulations are available for in vitro diagnostic (IVD) use in select regions.

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

Applications

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

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



1 Prepare Cells

Process cells by either:

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

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

2 Add Cells to MethoCult™

Add cells to MethoCult™ and vortex.

3 Plate and Incubate

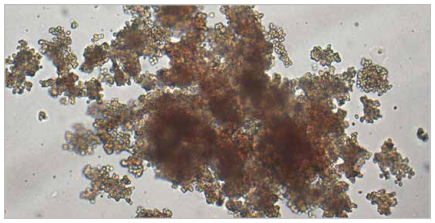
Dispense cells into 35 mm culture dishes or SmartDish™ using a syringe and blunt-end needle. Incubate human cells for 7-14 days, or mouse cells for 7-12 days, in a humidified incubator at 37°C and 5% CO_2.

4 Count Colonies

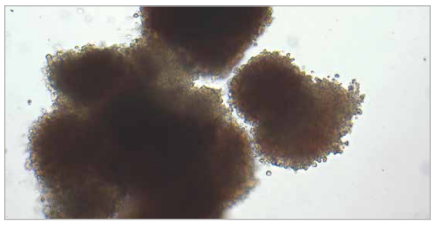
Count and evaluate colony types manually, using inverted microscope and STEMgrid™-6 scoring grid or automatically, using STEMvision™.

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

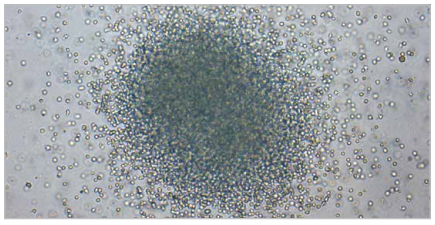
Figure 16. Colony-Forming Unit (CFU) Assay Procedure



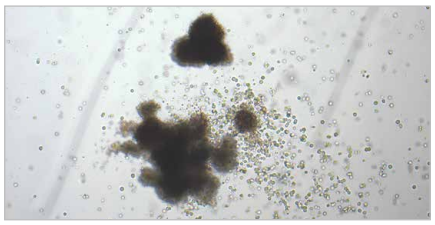
Human BFU-E (high power)



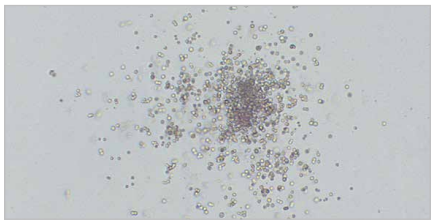
Human BFU-E (high power)



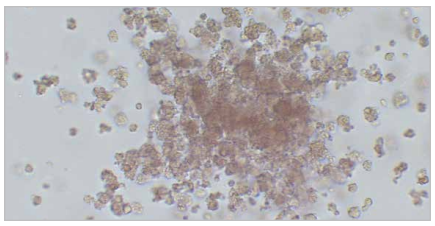
Human CFU-GM (high power)



Human CFU-GEMM (medium power)



Mouse CFU-M (low power)



Mouse BFU-E (high power)

Figure 17. Images of Human and Mouse Colonies Visualized on an Inverted Microscope

MethoCult™ Media for Human and Mouse Cells

MethoCult™ Media for Human Cells

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

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

IVD Registered MethoCult™ Media for Human Cells*

METHOCULT™ PRODUCT	CATALOG #	UNIT SIZE	GROWTH FACTORS**	APPLICATIONS
GF H84434	84434 84444	100 mL 24 x 3 mL	rhSCF, rhIL-3, rhEPO, rhG-CSF, rhGM-CSF	• Detection and quantification of human hematopoietic progenitor cells (BFU-E, CFU-E, CFU-GM, CFU-G, CFU-M, CFU-GEMM) in BM, MPB, PB, and CB using the CFU assay • Compatible with STEMvision™
GF H84534	84534 84544	100 mL 24 x 3 mL	rhSCF, rhIL-3, rhG-CSF, rhGM-CSF	• Detection and quantification of human hematopoietic progenitor cells (CFU-GM, CFU-G, CFU-M) in BM, MPB, PB, and CB using the CFU assay • Compatible with STEMvision™
GF H84435	84435 84445	100 mL 24 x 3 mL	rhSCF, rhIL-3, rhIL-6, rhEPO, rhG-CSF, rhGM-CSF	Detection and quantification of human hematopoietic progenitor cells (BFU-E, CFU-E, CFU-GM, CFU-G, CFU-M, CFU-GEMM) in BM, MPB, PB, and CB using the CFU assay
GF H84535	84535 84545	100 mL 24 x 3 mL	rhSCF, rhIL-3, rhIL-6, rhG-CSF, rhGM-CSF	Detection and quantification of human hematopoietic progenitor cells (CFU-GM, CFU-G, CFU-M) in BM, MPB, PB, and CB using the CFU assay
Cell Wash Medium	87700	100 mL	n/a	Cell diluent and washing medium

*Registered as In Vitro Diagnostic (IVD) medical devices in select regions. For more information, visit **www.stemcell.com/regulated-products**
**All IVD MethoCult™ media for human cells contain MC, FBS, and BSA.

MethoCult™ Media for Mouse Cells

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

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

STEMvision™

Automated and Standardized Counting of CFU Assays

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

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

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

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

SYSTEM IS SUPPLIED WITH:

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

REQUIRED REAGENTS:

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

CAPACITY:

- One 6-well SmartDish™ per run

STEMvision™ Instrument

Why Use STEMvision™ ?

ACCURACY. Faster and more accurate colony counts than with manual counting.

STANDARDIZE. Eliminate the subjectivity associated with manual colony counting.

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

Software for Human Assays

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

Software for Mouse Assays

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

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

Human Cord Blood CFU Assays

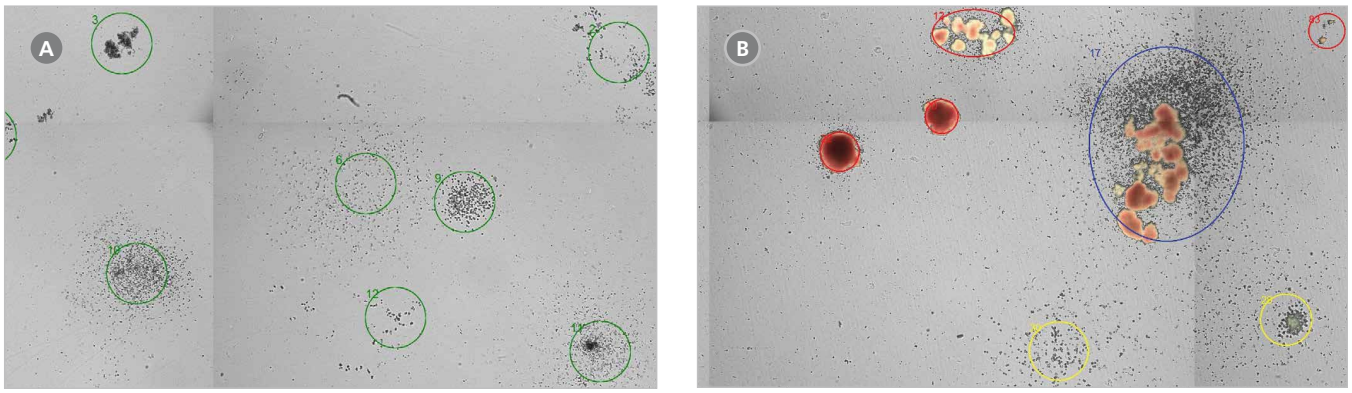


Figure 18. Representative STEMvision™ Images Showing Colonies Derived from Human Cord Blood Progenitor Cells After 7 Days of Culture in MethoCult™ Express or After 14 Days of Culture in MethoCult™ Optimum

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

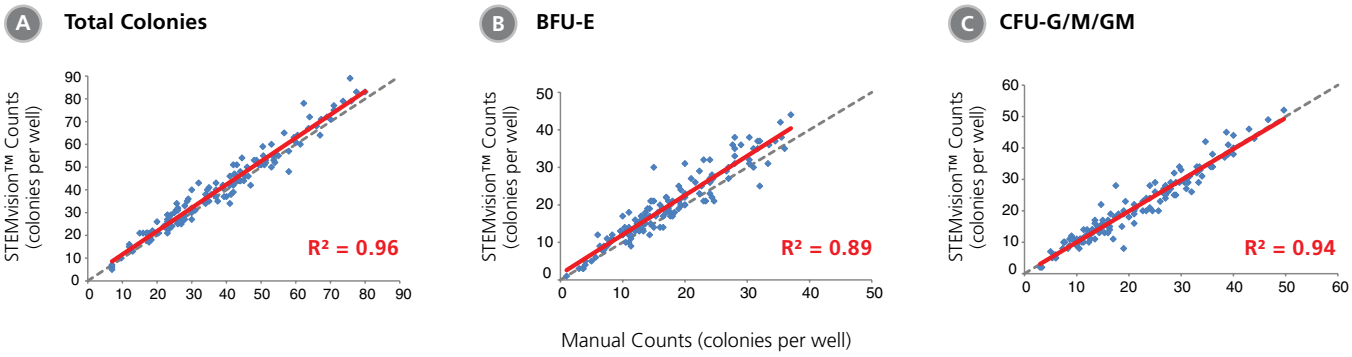


Figure 19. STEMvision™ Automated Counts of Total, Erythroid (BFU-E), and Myeloid (CFU-G/M/GM) Colonies are Highly Correlated to Manual Counts of 14-Day CB CFU Assays

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

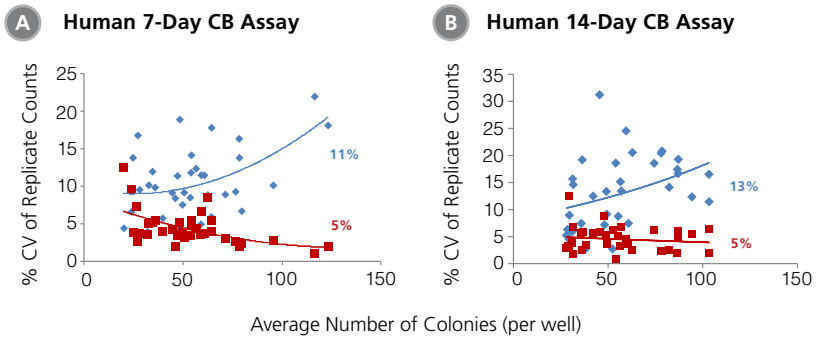


Figure 20. STEMvision™ Automated Colony Counting of 7-Day and 14-Day CB CFU Assays is More Reproducible than Manual Counting

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

Mouse Bone Marrow CFU Assays

MethoCult™ GF M3434

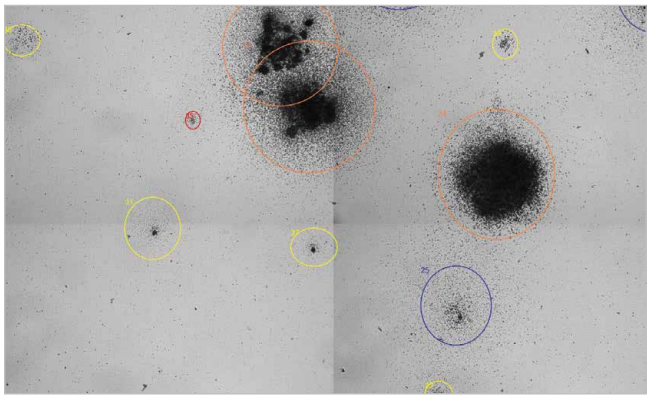


Figure 21. Representative STEMvision™ Images Showing Colonies Derived from Mouse Bone Marrow Progenitor Cells After 12 Days of Culture in MethoCult™ GF M3434 Medium

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

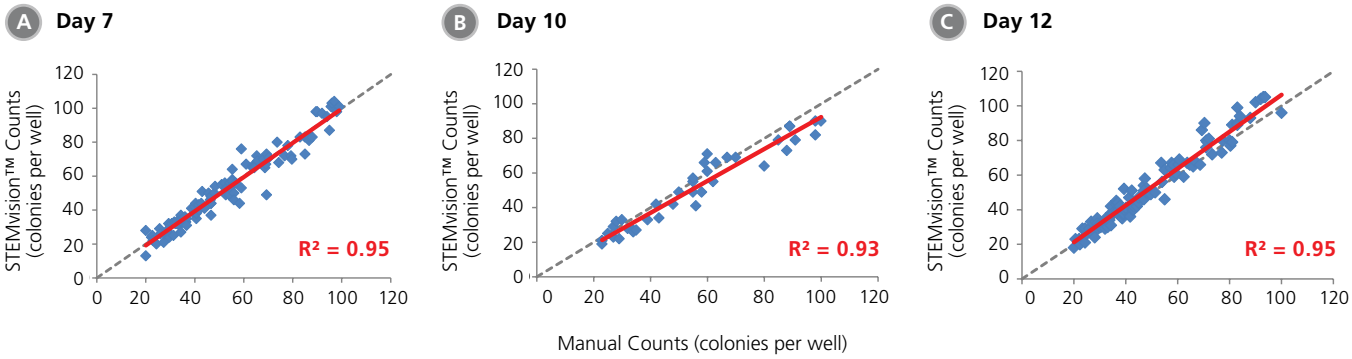


Figure 22. STEMvision™ Automated Counting of Total (Myeloid plus Erythroid) Colonies on Day 7, 10, and 12 is Highly Correlated to Manual Counts of CFU Assays Using Mouse Bone Marrow Cells

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

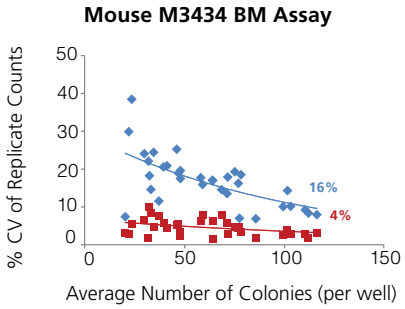


Figure 23. STEMvision™ Automated Colony Counting of CFU Assays with Mouse Cells is More Reproducible than Manual Counting

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

SmartDish™ and STEMgrid™-6

Meniscus-Free Cultureware for More Accurate Counting of Hematopoietic Colonies

When the CFU assay is performed using traditional cultureware, medium forms a meniscus where it meets the dish. The greater medium depth in the meniscus results in a greater proportion of colonies forming around the periphery (Figure 24A) where optical distortion can make it challenging to identify colonies (Figures 25A and 25C). This can reduce the accuracy of colony counting (i.e. result in undercounting of CFUs).

SmartDish™ 6-well culture plates have been designed to enable accurate and reproducible colony counting by preventing the formation of a meniscus. This allows for more uniform distribution of culture medium (Figure 24B) which results in a more even distribution of colonies throughout the entire 35 mm well. Additionally, the absence of the meniscus reduces optical distortion so that colonies present at the edge of the dish can be more easily counted (Figures 25B and 25D).

SmartDish™ has been designed to work with standard inverted microscopes when performing manual colony counts (Figure 25B), as well as with the STEMvision™ instrument for automated counting of hematopoietic CFU assays (Figure 25D). For manual counting of hematopoietic CFU assays, SmartDish™ is used with STEMgrid™-6, a detachable counting grid that facilitates navigation throughout the culture and also divides it into four quadrants for partial counting if desired.

SmartDish™ Meniscus-Free Cultureware

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

RECOMMENDED FOR:

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

STEMgrid™-6 Counting Grid

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

RECOMMENDED FOR:

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

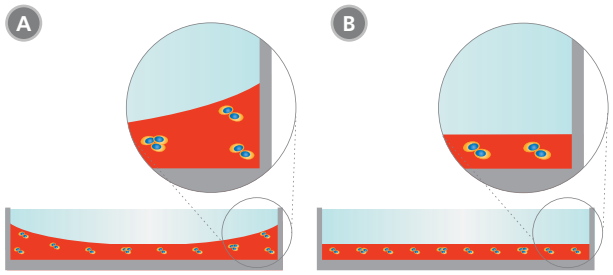
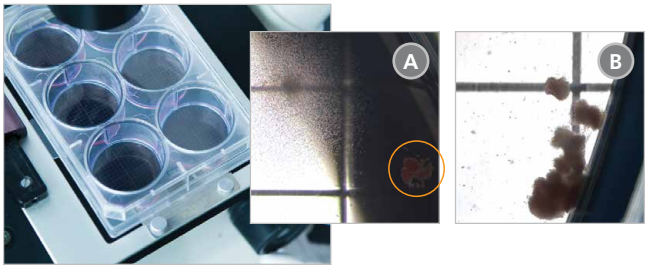


Figure 24. Schematic Illustration of Medium and Colony Distribution in Standard and SmartDish™ Cultureware

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

Manual Counting of Hematopoietic CFU Assays



Automated Counting of Hematopoietic CFU Assays

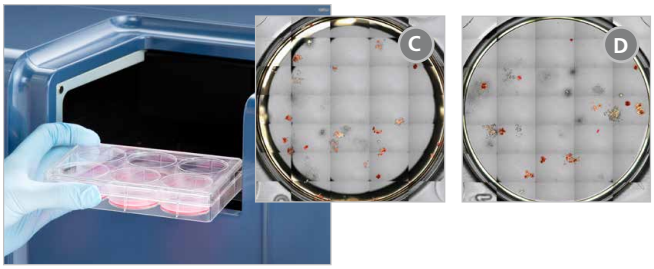


Figure 25. 14-Day Human Cord Blood CFU Assays Performed in Standard Non-Treated and SmartDish™ 6-Well Culture Plates

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

MegaCult™ Collagen-Based Media

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

Human and Mouse MegaCult™ Media and Staining Kits

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

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

Why Use Collagen-Based Media?

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

Why Use MegaCult™ ?

CONVENIENT. Culture and staining can be performed on one slide and evaluated immediately or stored for examination at a later time.

OPTIMIZED. Serum-free MegaCult™ medium is designed specifically for the quantitation of human megakaryocytic progenitor cells.[78,79]

FLEXIBLE. Serum-free MegaCult™ medium, without cytokines, allows for the addition of desired cytokines for the assay of human or mouse megakaryocytic progenitor cells.

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

Antibodies and ELISA Kits for HSPC Research

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

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

Antibodies for Human Hematopoietic Cell Research

ANTIGEN	CLONE	ISOTYPE	CONJUGATE	CATALOG #
CD3	SK7	Mouse IgG_1, kappa	FITC	60127
	UCHT1	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP, PerCP-Cy5.5	60011
CD11b	ICRF44	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60040
CD14	MoP9	Mouse IgG_{2b}, kappa	FITC	60124
	M5E2	Mouse IgG_{2a}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60004
CD16	3G8	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60041
CD19	HIB19	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60005
	6D5	Rat IgG_{2a}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, Pacific Blue™, PE	60006
CD20	2H7	Mouse IgG_{2b}, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60008
CD34	8G12	Mouse IgG_1, kappa	APC, FITC, PE	60121
	563	Mouse IgG_1, kappa	PE	60119
	581	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60013
CD38	AT-1	Mouse IgG_1, kappa	FITC	60131
	HIT2	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60014
CD45	2D1	Mouse IgG_1, kappa	FITC	60123
	HI30	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, Pacific Blue™, PE, PerCP-Cy5.5	60018
CD71 (Transferrin Receptor)	OKT9	Mouse IgG_1, kappa	Unconjugated, APC, Biotin, FITC, PE	60106
CD90 (Thy-1)	5E10	Mouse IgG_1, kappa	Unconjugated, APC, Biotin, FITC, PE, PerCP-Cy5.5	60045
CD105	43A3105	Mouse IgG_1, kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60039
CD117 (c-Kit)	104D2	Mouse IgG_1, kappa	Unconjugated, APC, Biotin, PE	60087
CD123 (IL-3Rα)	6H6	Mouse IgG_1, kappa	Unconjugated, APC, Biotin, FITC, PE, PerCP-Cy5.5	60110
CD235a (Glycophorin A)	2B7	Mouse IgG_1, kappa	FITC	60152
CD235ab (Glycophorin A/B)	HIR2	Mouse IgG_{2b}, kappa	Unconjugated, APC, Biotin, FITC, PE	60111

Antibodies for Mouse Hematopoietic Cell Research

ANTIGEN	CLONE	ISOTYPE	CONJUGATE	CATALOG #
CD3e	145-2C11	Hamster (Armenian) $IgG_{1,}$ kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60015
CD4	RM4-5	Rat $IgG_{2a,}$ kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60017
	RM4-4	Rat $IgG_{2b,}$ kappa	APC, Biotin, FITC, PE	60029
CD8a	53-6.7	Rat $IgG_{2a,}$ kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60023
CD11b	M1/70	Rat $IgG_{2b,}$ kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, Pacific Blue™, PE, PerCP-Cy5.5	60001
CD19	6D5	Rat $IgG_{2a,}$ kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, Pacific Blue™, PE	60006
CD43	R2/60	Rat $IgM_{,}$ kappa	Biotin	60042
CD45.1	A20	Rat $IgG_{2a,}$ kappa	Biotin	60117
CD45.2	104	Rat $IgG_{2a,}$ kappa	Biotin	60118
CD45	30-F11	Rat $IgG_{2b,}$ kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60030
CD48 (SLAMF2)	HM48-1	Hamster (Armenian) IgG1, lambda3	APC	60162
CD45R (B220)	RA3-6B2	Rat $IgG_{2a,}$ kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, Pacific Blue™, PE, PerCP-Cy5.5	60019
CD117 (c-Kit)	2B8	Rat $IgG_{2b,}$ kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60025
	ACK2	Rat $IgG_{2b,}$ kappa	Unconjugated, Alexa Fluor® 488, APC, FITC, PE	60034
CD150 (SLAM)	TC15-12F12.2	Rat $IgG_{2a,}$ lambda	Unconjugated, Alexa Fluor® 488, APC, Biotin, PE, PE-Cyanine7	60036
EPCR (CD201)	RMEPCR1560	Rat $IgG_{2b,}$ kappa	Unconjugated, Biotin, FITC, PE	60038
Gr-1 (Ly-6G/Ly-6C)	RB6-8C5	Rat $IgG_{2b,}$ kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60028
Ly-6G	1A8	Rat $IgG_{2a,}$ kappa	Unconjugated, APC, Biotin, FITC, Pacific Blue™, PE, PerCP-Cy5.5	60031
Sca1 (Ly-6A/E)	E13-161.7	Rat $IgG_{2a,}$ kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE	60032
Rhesus	T3G6	Mouse $IgG_{1,}$ kappa	Unconjugated	60132
	T4G6	Mouse $IgG_{1,}$ kappa		60133
TER119	TER-119	Rat $IgG_{2b,}$ kappa	Unconjugated, Alexa Fluor® 488, APC, Biotin, FITC, PE, PerCP-Cy5.5	60033

Detection Assays for Cord Blood Potency Testing

ALDH^{br} Assay Kit

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

Product Information

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

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

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

Tissue Culture Reagents and Supplies

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

Tissue Culture Media

PRODUCT NAME	CATALOG #	UNIT SIZE
DMEM with 4500 mg/L D-glucose	36250	500 mL
DMEM with 1000 mg/L D-glucose	36253	500 mL
DMEM/F-12	36254	500 mL
Iscove's MDM (IMDM)	36150	500 mL
IMDM with 2% FBS	07700	100 mL
McCoy's 5A Medium (Modified)	36350	500 mL
MEM Alpha with Nucleosides	36450	500 mL
MEM	36550	500 mL

Balanced Salt Solution

PRODUCT NAME	CATALOG #	UNIT SIZE
D-PBS	37350	500 mL
D-PBS, 10X	37354	500 mL
D-PBS with 2% FBS	07905	500 mL
HBSS, Ca^{++} & Mg^{++} free	37250	500 mL
HBSS, without Phenol Red	37150	500 mL

Supplements

PRODUCT NAME	CATALOG #	UNIT SIZE
Heparin	07980	2 mL
Hydrocortisone Powder	07904	100 mg
L-glutamine, 200 mM	07100	100 mL
MEM Non-Essential Amino Acids, 100X	07600	100 mL
Human Low-Density Lipoproteins (LDL)	02698	5 mg

Enzymes

PRODUCT NAME	CATALOG #	UNIT SIZE
Collagenase	07902	5 mL
DNase I, 1 mg/mL	07900	1 mL
Trypsin in Citrate Saline	07400	100 mL
Trypsin-EDTA	07901	500 mL

Miscellaneous Tissue Culture Reagents

PRODUCT NAME	CATALOG #	UNIT SIZE
3% Acetic Acid with Methylene Blue	07060	100 mL
Ammonium Chloride Solution	07800 07850	100 mL 500 mL
Trypan Blue	07050	100 mL

Tissue Culture Dishes & Slides

PRODUCT NAME	CATALOG #	UNIT SIZE
Outer Dishes for CFU Assays in 35 mm Dishes or SmartDish™ Plates		
Tissue Culture-Treated Dishes	38046	500 Dishes
Corning R 245 mm Square Dish, Non-Treated	38020	4/pack 16/case
Non-Adherent Culture Dishes *Recommended for CFU assays using MethoCult™		
35 mm Diameter Dishes	27100 27150	10/pack 500/case
Other Dishes and Slides		
Double Chamber Slides	04963	48 double chamber slides, filter cards and spacers
60 mm Gridded Scoring Dishes	27500	5/pack

Miscellaneous Tissue Culture Supplies

PRODUCT NAME	CATALOG #	UNIT SIZE
Blunt-End Needles	28110 28120	100/pack 2000/case
Reversible Strainer, 37 µm	27305	50/case
Tissue Dissociation Flask, glass, 250 mL	27300	1 flask
StemSpan™ Spinner Flask, glass, 50 mL	28800	1 flask
Hypoxia Chamber	27310	1 chamber

Serum Substitutes

PRODUCT NAME	CATALOG #	UNIT SIZE
10% Bovine Serum Albumin (BSA) in Iscove's MDM	09300	100 mL
BIT 9500 Serum Substitute, pre-screened lots of BSA, insulin, and transferrin in Iscove's MDM	09500	100 mL

Proficiency Testing Programs and Quality Control Kits

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

Proficiency Testing Programs

Evaluate your ability to perform all steps of the CFU assay from thawing samples to plating cells in methylcellulose medium, in our Proficiency Testing programs. Participants are provided with a cell sample, MethoCult™ medium, additional reagents and supplies, and detailed instructions required to perform the CFU assay. The results from all participants are analyzed and compared to the cohort mean (Figure 26) according to guidelines outlined in ISO 13528.

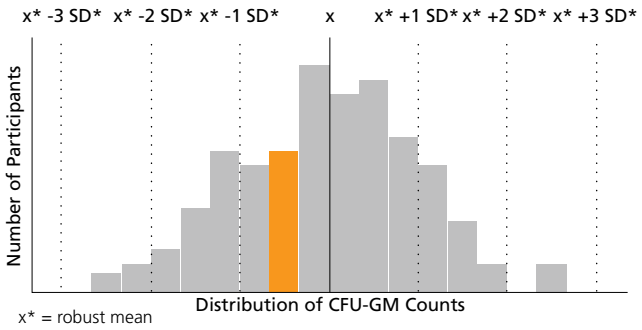


Figure 26. Example Participant Data

Proficiency Testing Supplies

PROFICIENCY TESTING PROGRAM	CATALOG #
Frozen Human Bone Marrow (BM)	00602
Fresh Cord Blood (CBH)	00606
Frozen Cord Blood (CBZ)	00608

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

Quality Control Kits

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

QC Kit Supplies

QC KIT	CATALOG #	APPLICATION
Human Bone Marrow (BM)	00650	Monitoring reproducibility in performing BM CFU assays
Human Cord Blood (CB)	00651	Monitoring reproducibility in performing CB CFU assays

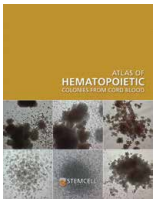
Training Courses and Instructional Materials

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

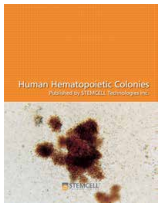
Training Courses for Hematopoietic Progenitor Assays

COURSE NAME	CATALOG #	COURSE DESCRIPTION
Standardization of the Hematopoietic Progenitor Assay	00215	This 2-day course focuses on the standardization of the colony-forming unit (CFU) assay for human samples • Gain hands-on experience in the assay set-up, identification, and enumeration of hematopoietic progenitor cells using MethoCult™
Applications of the Hematopoietic Progenitor Assay	00217	This 3-day course focuses on hematopoietic progenitor assays for human, mouse, rat, and canine samples with an emphasis on applying the CFU assay to your research. Learn to: • Effectively design in vitro and in vivo assays to understand the effects of biological pathways on hematopoietic homeostasis • Evaluate the effect of compounds on blood and bone marrow using various mouse models • Use the CFU assay in drug development and predictive toxicity testing

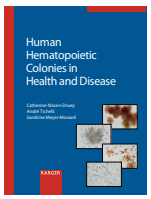
STEMCELL Technologies also offers customized training courses, which can be highly beneficial to address specific research challenges or to standardize technical processes. Custom training packages provide your team with personalized instruction from a technical specialist at your facility or at our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



Catalog #29940



Catalog #28700



Catalog #28760

Instructional Materials

PRODUCT NAME	CATALOG #	DESCRIPTION
Cord Blood Colony Atlas	29940	• Detailed color images of hematopoietic colonies derived from human umbilical cord blood progenitor cells grown in methylcellulose-based media
Human Hematopoietic Colony Atlas	28700	• A guide for the process of identifying and evaluating colonies derived from human hematopoietic progenitor cells grown in methylcellulose-based media
Human Hematopoietic Colonies in Health and Disease	28760	• Colony-morphology from healthy individuals and patients with hematological disorders are illustrated in 230 detailed, color photographs

Technical Manuals for Hematopoietic Progenitor Assays

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

Contract Assay Services

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

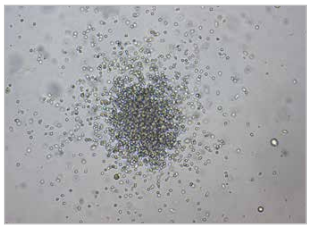
Stem and Progenitor Cell Assays

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

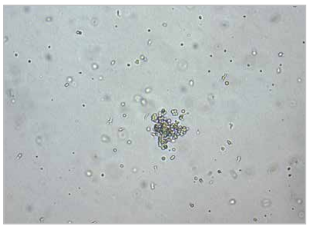
- CFU assays have been shown to be important in the evaluation of potential inhibitory or stimulatory effects of a variety of compounds on hematopoietic and mesenchymal progenitor cells[72,80-88]
- CFU assays can be used to assess proliferation and differentiation of hematopoietic progenitor cells to determine IC_{50} and IC_{90} values
- CFU assays for myeloid progenitor cells have been validated for the determination of maximum tolerated dose by the European Centre for the Validation of Alternative Methods (ECVAM)
- Assays for CFU-GM and CFU-Mk have been shown to be predictive of clinical outcomes such as neutropenia and thrombocytopenia[72,81,82]

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

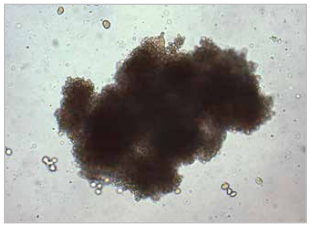
- Assess hematopoietic stem cell mobilization into the peripheral blood
- Determine kinetics of hematopoietic progenitor cell recovery following induction of myelosuppression



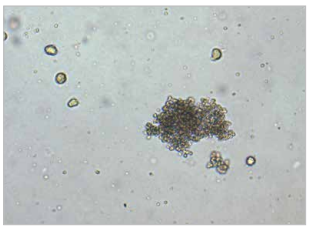
CFU-GM



CFU-GM with Inhibitory Compound



BFU-E



BFU-E with Inhibitory Compound

Figure 27. Effect of the Addition of an Inhibitory Compound on the Morphology of Human Bone Marrow CFU-GM and BFU-E Colonies

Interested in Contract Assay Services?

To learn more about CAS at STEMCELL Technologies, visit **www.contractassay.com** or contact us at **contractassay@stemcell.com** to find out how we can help you meet your goals.

Additional Information for CFU Assays

Recommended Plating Concentrations for CFU Assays of Human Cells

CELL SOURCE	CELLS PER 35 MM DISH
Bone Marrow – Ammonium Chloride Treated	2×10^4 - 1×10^5
Bone Marrow – Mononuclear Cells*	1 - 5×10^4
Cord Blood – Mononuclear Cells*	5×10^3 - 2×10^4
Normal Peripheral Blood – Mononuclear Cells*	1 - 4×10^5
Mobilized Peripheral Blood	1 - 5×10^4
Lineage-Depleted $CD34^+$ Cell Enriched BM, CB, MPB	0.5 - 2×10^3**
Purified $CD34^+$ cells (BM, CB, MPB)	0.15 - 1×10^3**

*Mononuclear cells (MNCs) isolated by density-based cell separation (light density, 1.077 g/mL).
**Dependent on $CD34^+$ cell purity. Generally, 10 - 20% of $CD34^+$ cells form colonies.

Typical Progenitor Frequencies in Normal Human Samples

CELL SOURCE	PROGENITOR TYPE			
	CFU-E	BFU-E	CFU-GM	CFU-GEMM
Bone Marrow – Ammonium Chloride Treated per 10^5 cells (n = 50)	31 (1 - 78)	115 (1 - 251)	100 (30 - 170)	5 (1 - 15)
Bone Marrow – Mononuclear Cells* per 10^5 cells (n = 17)	188 (1 - 506)	175 (1 - 477)	408 (1 - 990)	10 (1 - 30)
Bone Marrow – $CD34^+$ Cell Enriched per 10^3 cells (n = 15)	30 (1 - 59)	34 (1 - 74)	54 (7 - 101)	2 (1 - 5)
Cord Blood – Mononuclear Cells* per 10^5 cells (n = 16)	9 (1 - 48)	104 (1 - 310)	115 (1 - 303)	25 (1 - 59)
Normal Peripheral Blood – Mononuclear Cells* per 10^5 cells (n = 30)	2 (1 - 10)	30 (1 - 62)	9 (1 - 18)	2 (1 - 5)
Mobilized Peripheral Blood per 10^5 cells (n = 19)	8 (1 - 27)	121 (1 - 257)	111 (1 - 257)	23 (1 - 67)

CFU numbers were determined using MethoCult™ H4434 Classic. Values are expressed as means; the range is defined by mean ± 2 standard deviations.
*Mononuclear cells (MNCs) isolated by density-based cell separation (light density, 1.077 g/mL).

Recommended Plating Concentrations for CFU Assays of Mouse Cells

CELL SOURCE	CFU ASSAY	METHOCULT™ MEDIUM	CELLS PER 35 MM DISH
Mouse Bone Marrow	CFU-E, Mature BFU-E	M3334	1 - 2×10^5
Mouse Bone Marrow	BFU-E, CFU-GM, CFU-GEMM	M3434	2×10^4
Mouse Bone Marrow	CFU-pre-B	M3630	1 - 2×10^5
Adult Spleen and Peripheral Blood	BFU-E, CFU-GM, CFU-GEMM	M3434	2×10^5
Fetal Liver (12 - 15 dpc)	BFU-E, CFU-GM, CFU-GEMM	M3434	2×10^4
Lin^- *	BFU-E, CFU-GM, CFU-GEMM	M3434	1×10^3
Yolk Sac	BFU-E, CFU-GM, CFU-GEMM	M3434	5×10^4
Mouse Bone Marrow	BFU-E	M3436	3 - 8×10^4

The plating concentrations were established using C57BL/6 strains. For other strains, transgenic or treated mice, plate cells at 2 - 3 cell densities to establish optimal plating concentrations. Cell purification steps are generally not necessary. RBCs can be removed from spleen and peripheral blood samples by ammonium chloride lysis (Catalog# 07800/07850).
*Lin^- cells from mouse bone marrow and fetal liver are isolated using EasySep™ Mouse Progenitor Cell Isolation Kit (Catalog #19856).

Typical Progenitor Frequencies in Normal Mouse Bone Marrow

PROGENITOR	METHOCULT™ MEDIUM	CFU PER 106 BONE MARROW CELLS
CFU-E	M3334	1700 ± 400
BFU-E CFU-GEMM CFU-GM	M3434	400 ± 130 170 ± 70 3200 ± 800
CFU-Pre-B	M3630	1050 ± 30
BFU-E	M3436	720 ± 460

CFU-E: colony-forming unit – erythroid; BFU-E: burst-forming unit – erythroid; CFU-GEMM: colony-forming unit – granulocyte, erythrocyte, macrophage, megakaryocyte; CFU-GM: colony-forming unit – granulocyte/macrophage; CFU-Pre-B: colony-forming unit – pre-B.
Assays performed using 6 - 12 week old C57Bl/6 mice. Values are expressed as means; the range is defined by mean ± 2 standard deviations.

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1. Delaney C et al. (2010) Nat Med 16(2): 232–6.
2. Cutler C et al. (2013) Blood 122(17): 3074–81.
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DOC#29054 VERSION 6.1.0 SEPT 2019