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## A Complete Set of Tools for the CFU Assay

STEMCELL Technologies, Inc. offers a comprehensive line of products to determine the number of CFUs in cord blood samples. STEMCELL Technologies' Quality Management System is certified to ISO 13485 Medical Device Standards.

Please visit us at www.stemcell.com for additional information.

# Standardized Counting of Colony-Forming Unit (CFU) Assays

## for Human Hematopoietic Cells







The colony-forming unit (CFU) assay is the gold standard in vitro functional assay for measuring the number of progenitor cells in human hematopoietic cell populations. The CFU assay has numerous applications for basic and clinical research in hematopoiesis, and in hematopoietic stem cell transplantation.

Historically, the CFU assay has been performed by culturing hematopoietic cells in MethoCult™ medium and counting the number of colonies produced by different sub-types of CFUs 14 days later using an inverted microscope. Colonies produced by different types of lineage-restricted and multi-potential progenitor cells (i.e. colony-forming unit-erythroid (CFU-E), burst-forming unit-erythroid (BFU-E), colony-forming unit-granulocyte, macrophage (CFU-GM) and colony-forming unit-granulocyte, erythrocyte, macrophage and megakaryocyte (CFU-GEMM)), are identified and scored on the basis of well-defined morphological criteria. However, accurate counting of colony types can be challenging for individuals with limited experience. Manual counting of CFU assays is also time consuming and costly for laboratories that perform large numbers of assays each day.

STEMvision<sup>TM</sup> is a bench-top instrument and computer system designed specifically for automated imaging and counting of hematopoietic colonies in the CFU assay. This system has been optimized for use with MethoCult<sup>TM</sup> media and meniscus-free SmartDish<sup>TM</sup> cultureware (Figure 1). The use of this standardized platform significantly improves the accuracy and reproducibility of the human CFU assay.

Instead of manually identifying and counting colonies using a microscope, the user simply loads a SmartDish™ culture plate into STEMvision™ and the instrument performs these functions. STEMvision™ captures an image of each 35 mm well in approximately 1 minute and then uses highly sophisticated image analysis software to identify and classify each colony into the four major sub-types produced by CFU-E, BFU-E, CFU-G/M/GM and CFU-GEMM.

Users can choose to image and analyze each 35 mm well in a single step. Alternatively, for high-throughput processing, multiple dishes can be imaged sequentially and then analysed, in approximately 1 minute per well, overnight or at a later time, if desired.

# A Complete Workflow For Human Hematopoietic CFU Assays:

- Primary human hematopoietic cells
- EasySep™ and RoboSep™ for isolation of hematopoietic stem and progenitor cells
- Reagents for removing red blood cells from samples
- MethoCult™ Express and MethoCult™ Optimum media for 7- and 14-day CFU assays, respectively
- SmartDish™ meniscus-free cultureware for more reliable colony counting
- STEMvision™ for automated and standardized colony counting
- Analysis Packages for counting the four major CFU subtypes in human CB, BM and MPB
- Proficiency testing programs

Different Analysis Packages have been developed to accurately count CFUs in 14-day assays of human umbilical cord blood (CB), bone marrow (BM) and mobilized peripheral blood (MPB), using MethoCult<sup>TM</sup> Optimum. HetaSep<sup>TM</sup> has been developed to remove red blood cells (RBCs) from fresh CB, BM and MPB samples prior to CFU analysis, while other toosl such as ErythroClear<sup>TM</sup> have been specifically developed for small volume fresh or frozen CB samples. RBC depletion improves the accuracy of the CFU assay whether colonies are counted manually or using STEMvision<sup>TM</sup>. A faster CFU assay that uses MethoCult<sup>TM</sup> Express has also been developed specifically for CB banks, enabling the number of CFUs in CB units to be measured in only 7 days. This can allow results to be obtained in sufficient time to inform decision-making around CB transplantation.

# The CFU Assay Workflow

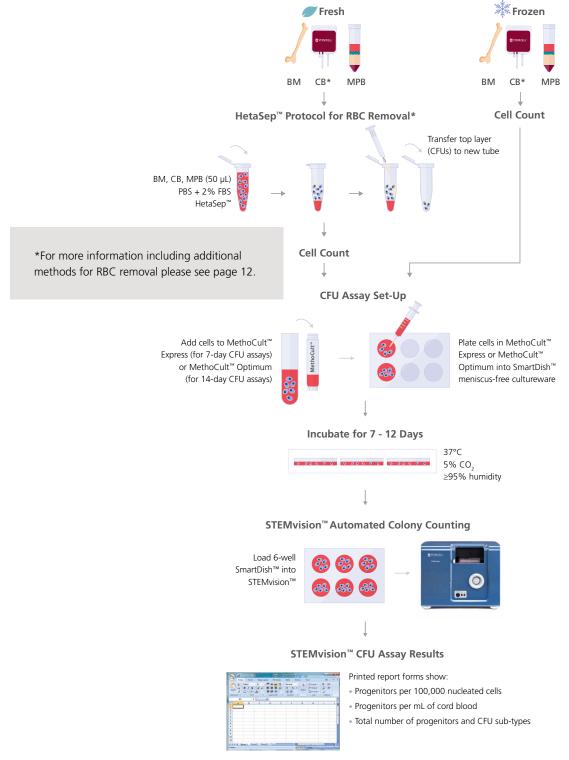


Figure 1. A Typical CFU Assay Workflow Incorporating STEMvision™ for Automated Counting of Hematopoietic Colonies

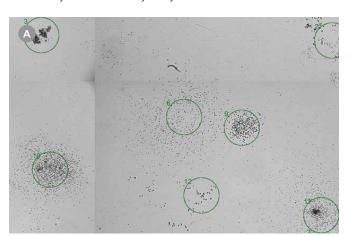
Red blood cells (RBCs) are removed from 50 µL of fresh cord blood (CB), bone marrow (BM) or mobilized peripheral blood (MPB) samples using HetaSep™. The ErythroClear™ Red Blood Cell Depletion Kit (Catalog #01739) is also available for RBC removal, designed for use with small volume samples of fresh or frozen cord blood. RBC removal is required for fresh samples and recommended for frozen samples as it decreases background and improves the resolution of colonies, allowing proper analysis by STEMvision™. Cells are then cultured in SmartDish™ containing the appropriate MethoCult™ medium, depending on the cell type and whether the CFU assay will be counted after 7 or 14 days. STEMvision™ acquires an image of each culture, and then classifies and counts the number of colonies produced by the four major subtypes of hematopoietic progenitor cells; CFU-E, BFU-E, CFU-G/M/GM and CFU-GEMM. STEMvision™ can generate a printed report of the CFU assay results documenting the frequency and total number of CFUs in the sample. For cord blood banks, an additional report can be produced for the family if desired.

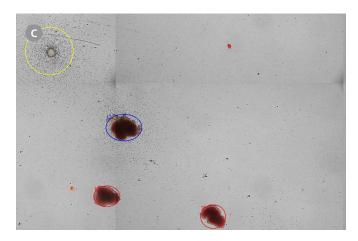
# **STEMvision**™

## Automated CFU Assay Imaging and Standardized Colony Counting

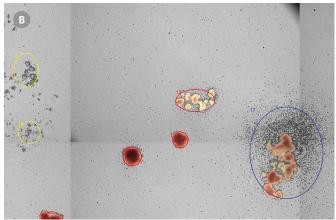
STEMvision™ is a bench-top instrument and computer system that automates and standardizes the process of counting hematopoietic colonies in the colony-forming unit (CFU) assay. STEMvision™ images each 35 mm well in approximately 1 minute, resulting in a high-resolution image. With our updated color instrument, colonies containing hemoglobinized cells are shown in their true red color. Sophisticated analysis software is then used to identify, classify and count the colonies produced by BFU-E, CFU-G/M/GM and CFU-GEMM progenitors, in approximately 1 minute per well (Figure 2).

By using an automated system to standardize colony identification and counting, cord blood (CB) banks can ensure that their CFU assay results are accurate and reproducible. STEMvision™ Analysis Packages have been developed to provide total CFU counts and colony classification in the conventional 14-day assay, or total CFU counts only in a faster 7-day assay of human CB cells.









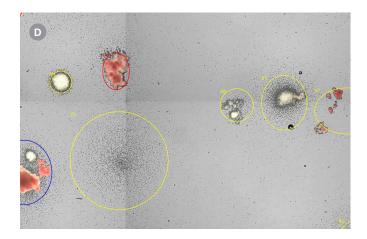


Figure 2. Representative STEMvision™ Images Showing Colonies Derived from CB Progenitors After 7 Days of Culture in MethoCult™ Express, and From CB, BM and MPB After 14 Days of Culture in MethoCult™ Optimum

These images have been analyzed by STEMvision™ Human (A) 7-Day and (B-D) 14-Day Analysis Packages. Green circles identify individual colonies in a 7-day CB CFU assay that scores (A) total CFUs only. Orange and red circles identify erythroid colonies (produced by CFU-E and BFU-E, respectively), yellow circles identify myeloid colonies (produced by CFU-GEMM) in (B) 14-day CB, (C) BM and (D) MPB CFU assays. Erythroid and mixed colonies that contain hemoglobinized cells are shown in true red color.

# **STEMvision™ Performance Data**

## Automated 14-Day CFU Assays of Human Cord Blood Cells

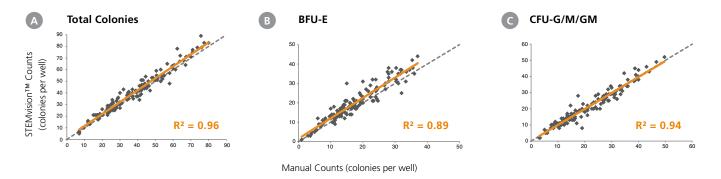


Figure 3. STEMvision™ Automated Counts of Total, Erythroid (BFU-E) and Myeloid (CFU-G/M/GM) Colonies are Highly Correlated to Manual Counts of 14-Day CB CFU Assays

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.

The mathematical equations and coefficients of determination (R<sup>2</sup>) that describe each data set (n=130 CFU assays) are as follows:

- Figure 3A: y=1.02x + 1.39; R2=0.96 for Total Colonies
- Figure 3B: y=1.05x + 1.53; R2=0.89 for BFU-E
- Figure 3C: y=0.99x + 0.13; R2=0.94 for CFU-G/M/GM

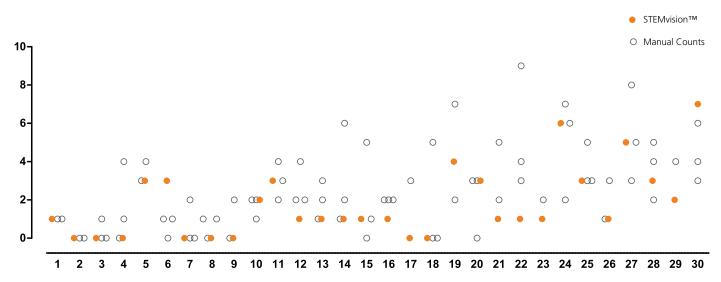


Figure 4. STEMvision™ Automated Counting of Mixed Colonies Falls Within the Range of Manual Counts of 14-Day CB CFU Assays

Thirty individual 14-day CB CFU assays were counted by three to seven people. The numbers of mixed (CFU-GEMM) colonies counted manually in each well are shown as open circles (n=80 total assay scores). Manual CFU-GEMM counts in most cultures varied between individual people. STEMvision™ counts of the same culture wells (red circles) provided a CFU-GEMM count that was typically within the range of manual counts.

# **STEMvision™ Performance Data**

## Automated 14-Day CFU Assays of Human Bone Marrow Cells

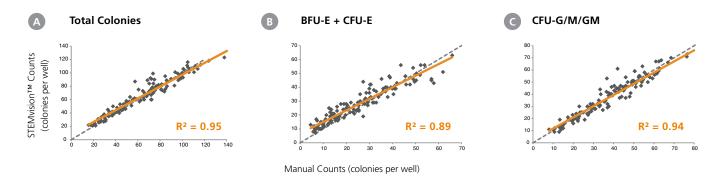


Figure 5. STEMvision™ Automated Scoring of Total, Erythroid (BFU-E + CFU-E) and Myeloid (CFU-G/M/GM) Colonies is Highly Correlated to Manual Counts of 14-Day BM CFU Assays

Cryopreserved BM cells were thawed, plated in MethoCult™ Optimum, cultured for 14 days, and the resulting colonies then scored both manually using an inverted microscope and automatically using STEMvision™. The BM Analysis Package can identify and count erythroid colonies produced by BFU-E and CFU-E separately, but these are combined in panel B. 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. The mathematical equations and coefficients of determination (R²) that describe each data set (n=120 CFU assays) are as follows:

- Figure 5A: y=0.88x + 8.79; R2=0.95 for Total Colonies
- Figure 5B: y=0.83x + 6.71; R2=0.89 for BFU-E + CFU-E
- Figure 5C: y=0.92x + 2.55; R2=0.94 for CFU-G/M/GM

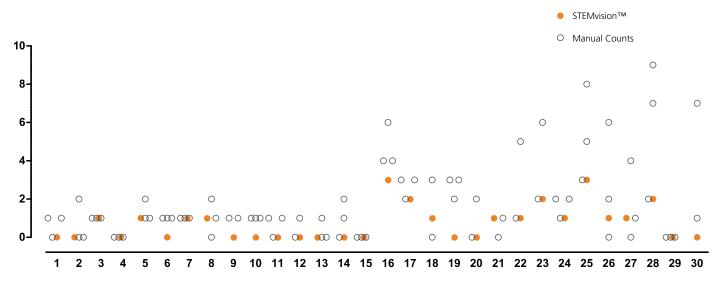


Figure 6. STEMvision™ Automated Scoring of Mixed Colonies Falls Within the Range of Manual Counts of 14-Day BM CFU Assays

Thirty individual 14-day BM CFU assays were counted by 3 to 7 people. The numbers of mixed (CFU-GEMM) colonies counted manually in each well is shown as open circles (n = 82 total assay scores). Manual CFU-GEMM counts in most cultures varied significantly between individual people. STEMvision $^{TM}$  counts of the same culture wells (red circles) provided a CFU-GEMM count that was typically within the range of manual counts.

# **STEMvision™ Performance Data**

## Automated 14-Day CFU Assays of Human Mobilized Peripheral Blood Cells

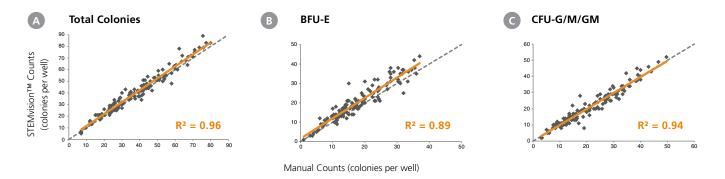


Figure 7. STEMvision™ Automated Counting of Total, Erythroid (BFU-E) and Myeloid (CFU-G/M/GM) Colonies is Highly Correlated to Manual Counts of 14-Day MPB CFU Assays

Cryopreserved MPB cells were thawed, plated in MethoCult™ Optimum, cultured for 14 days, and the resulting colonies then scored both manually using an inverted microscope and automatically using STEMvision™. 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. The mathematical equations and coefficients of determination (R²) that describe each data set (n=143 CFU assays) are as follows:

- Figure 7A: y=0.97x + 2.44; R2=0.97 for Total Colonies
- Figure 7B: y=0.96x + 3.74; R2=0.91 for BFU-E
- Figure 7C: y=0.96x + 0.90; R2=0.95 for CFU-G/M/GM

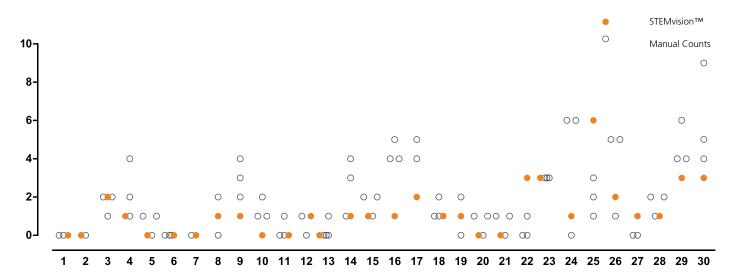


Figure 8. STEMvision™ Automated Scoring of Mixed Colonies Falls Within the Range of Manual Counts of 14-Day MPB CFU Assays

Thirty individual 14-day MPB CFU assays were counted by 3 to 7 people. The numbers of mixed (CFU-GEMM) colonies counted manually in each well are shown as open circles (n = 82 total assay scores). Manual CFU-GEMM counts in most cultures varied significantly between individual people. STEMvision<sup>TM</sup> counts of the same culture wells (red circles) provided a CFU-GEMM count that was typically within the range of manual counts.

# **STEMvision**™

## **Reduced Variability of CFU Counts**







Another important advantage of STEMvision<sup>TM</sup> for automated and standardized counting of hematopoietic colonies in human CFU assays is significantly improved reproducibility of assay results. The coefficient of variation in STEMvision<sup>TM</sup> colony counts is 2- to 3-fold lower in the recommended range of 20 - 80 colonies per 35 mm culture well than for counts produced by multiple technicians who manually count the same CFU assays.

The reduced variability of automated colony counts in 7-day and 14-day CFU assays of CB cells is shown in Figure 9 (A) and (B) respectively.

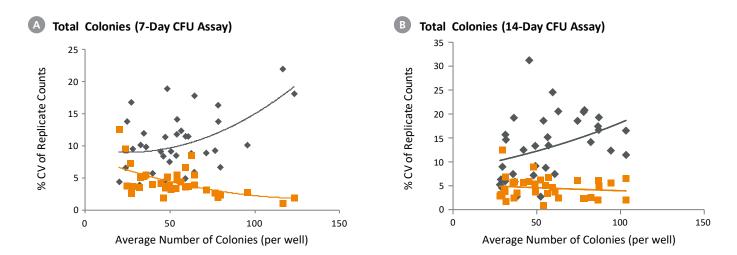


Figure 9. STEMvision™ Automated Colony Counting of 7-Day and 14-Day CB CFU Assays is More Reproducible Than Manual Counting

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 either manually by three to five different people (blue diamonds), or automatically using three to five 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%.

# **STEMvision™ CFU Assay Report Forms**

STEMvision™ produces two printed reports that detail information about the specific hematopoietic cell sample and the colony-forming unit (CFU) assay results (Figure 10). These reports provide critical functional information about the cell sample for the research or clinical laboratory's own records. In the case of cord blood (CB) banking, a second Parent Report form (not shown) can be produced for parents banking their child's CB if desired. The user-customizable information documented in these reports include:

- Laboratory address and contact information
- Patient and doctor demographic information
- Total number of viable progenitors in sample
- Sample and CFU assay tracking ID numbers
- CFU counts expressed per 100,000 nucleated cells or per mL of sample
- Assay counts for CFU-E, BFU-E, CFU-G/M/GM and CFU-GEMM are shown separately on 14-day CFU assay report forms
- Images of each replicate CFU assay displaying colonies and their classifications (colored circles).



Lab

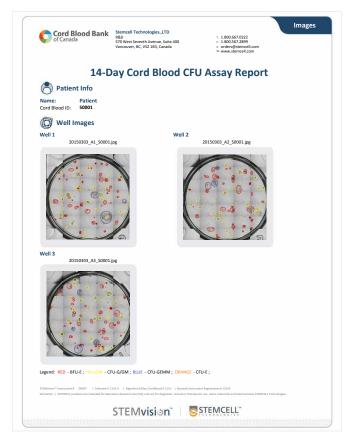


Figure 10. Sample STEMvision™ Lab Report for a 14-Day Cord Blood CFU Assay

# HetaSep™

## Depletion of Red Blood Cells from Fresh Blood Samples



HetaSep™

Product	Quantity	Catalog #
Lists Con TM (C. NA/All Distant)	20 mL	07806
HetaSep™ (6-Well Plates)	100 mL	07906

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.

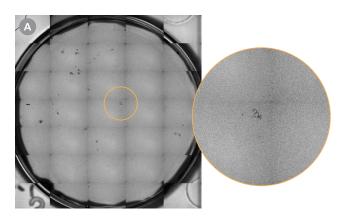
For more information, see the HetaSep™ Protocol Technical Bulletin (Document #29541) or visit **www.stemcell.com/hetasep\_protocol**.

## Why Use HetaSep™?

**ACCURATE.** Remove RBC background to increase the accuracy of colony counting.

**CONSISTENT.** Recover > 97% of colonies.

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



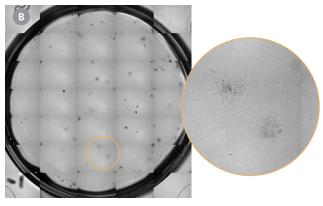


Figure 11. STEMvision™ Images of 7-Day CFU Assays of Fresh Cord Blood Samples Plated in MethoCult™ Express without and with Prior Removal of RBCs Using HetaSep™

(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.

# MethoCult™ Express & MethoCult™ Optimum

## Methylcellulose Media for 7-Day and 14-Day CFU Assays





**Figure 12.** Hematopoietic Colonies Grown in MethoCult™ Express, Visualized at Day 7 (Left) and Day 14 (Right) on an Inverted Microscope

STEMvision™ has been designed for use with MethoCult™ media in order to ensure optimal colony growth, counting and CFU assay accuracy. The 14-Day Analysis Packages (Catalog #22005, #22006, #22007) are used with MethoCult™ Optimum (Catalog #04034) medium which supports optimal growth of erythroid progenitors (CFU-E and BFU-E), granulocyte/macrophage progenitors (CFU-G, CFU-M and CFU-GM) and multi-potential granulocyte, erythrocyte, macrophage, megakaryocyte progenitors (CFU-GEMM) from human cord blood (CB), bone marrow (BM) and mobilized peripheral blood (MPB).

The Human Cord Blood 7-Day Analysis Package (Catalog #22001) is used with MethoCult™ Express (Catalog #04437) medium. MethoCult™ Express is formulated to accelerate the proliferation of human hematopoietic progenitor cells in CB and thus allows colonies to be counted after only 7 days; one week faster than with a conventional 14-day CFU assay. The total number of CFUs in CB measured after 7 days of culture in MethoCult™ Express correlates strongly with total CFU numbers measured after 14 days of culture in MethoCult™ Optimum. The 7-day CFU assay provides a simple method to determine the total number of viable and functional progenitor cells in a CB unit, without discriminating between the different sub-types of CFUs. Several clinical studies have shown that the total number of CFUs in a CB unit is the single parameter that correlates most strongly with engraftment outcomes following CB transplantation.<sup>1-5</sup>

MethoCult™ Optimum (Catalog #84434/84534) and MethoCult™ Express (Catalog #84437) are registered as In Vitro Diagnostic (IVD) medical devices in certain regions. Visit **www.stemcell.com/regulated-products** for a complete list of IVD products and their availability.

Outside of the registered regions MethoCult™ Optimum and MethoCult™ Express are available for research use only (RUO), not for therapeutic or diagnostic use.

Table 1. MethoCult™ Media Currently Validated for Automated Counting With STEMvision™\*

MethoCult™ Product	Catalog #	Size	Components			onents	Annline
			MC	FBS	BSA	Growth Factors	Applications
MethoCult™ Express (RUO)	04437	100 mL	✓	✓	<b>√</b>	Cytokines, including erythropoietin (EPO)	Measures the total number of CFUs in human CB in only 7 days
	04477	24 x 3 mL					
MethoCult™ Express (IVD)	84437	100 mL					
	84447	24 x 3 mL					
MethoCult™ Optimum (RUO)	04034	100 mL	✓	✓ <b>✓</b>	<b>√</b>		Supports growth of CFU-E, BFU-E, CFU-G/M/GM and CFU-GEMM in human CB, BM and MPB
	04044	24 x 3 mL					
MethoCult™ Optimum (IVD)	84434	100 mL					
	84444	24 x 3 mL					
MethoCult™ Optimum without EPO (RUO)	04035	100 mL		<b>√</b>	✓	Cytokines, no EPO	Supports growth of CFU-G, CFU-M and CFU-GM in human CB, BM and MPB
	04045	24 x 3 mL					
MethoCult™ Optimum without EPO (IVD)	84534	100 mL	<b>√</b>				
	84544	24 x 3 mL					

MC: methylcellulose; FBS: fetal bovine serum; BSA: bovine serum albumin; CB: cord blood; BM: bone marrow; MPB: mobilized peripheral blood \*Please contact Tech Support for more information.

## SmartDish™

## Meniscus-Free Cultureware for More Accurate Colony Counting

When a CFU assay is performed using traditional cultureware, a meniscus is formed between the culture medium and the sides of the culture dish. This meniscus results in greater medium depth at the periphery of the dish, leading to a higher proportion of colonies forming along its edges. Shadows and optical distortion caused by the meniscus can make it difficult to see these colonies at the edges of the dish (Figure 7A), reducing accuracy through possible undercounting of CFUs.

SmartDish™ 6-well culture plates are designed to improve the accuracy and reproducibility of colony counting by preventing the formation of a meniscus. This allows for an even distribution of culture medium, resulting in a more uniform distribution of colonies throughout the entire well. The absence of a meniscus also eliminates optical distortion so that colonies at the edge of each well can be more easily seen (Figure 7B). SmartDish™ cultureware is required for accurate and reproducible colony counting using STEMvision™.





### Why Use SmartDish™?

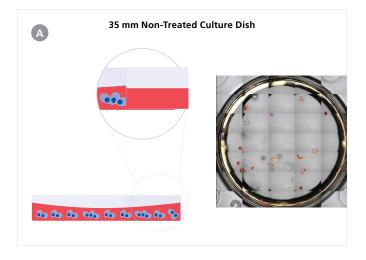
**CONSISTENT.** Results in an even distribution of colonies throughout each well.

**CLEAR.** No shadow or optical distortion at well edges.

**ACCURATE.** Colonies in SmartDish<sup>™</sup> plates may be counted using automated methods.

#### SmartDish™ Meniscus-Free Cultureware

Product	Quantity	Catalog #
SmartDish™ (6-Well Plates)	5/pack	27370
	50/pack	27371



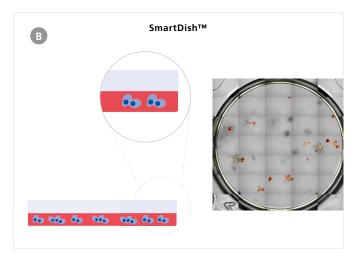


Figure 12. 14-Day CB CFU Assays Performed in Standard Non-Treated and SmartDish™ 6-Well Culture Plates

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

# **Product Information**

Product	Catalog #
STEMvision™ Instrument	22000/22000E
STEMvision™ Human Cord Blood 7-Day CFU Analysis Package	22001
STEMvision™ Human Cord Blood 14-Day CFU Analysis Package	22005
STEMvision™ Human Bone Marrow 14-Day CFU Analysis Package	22006
STEMvision™ Human Mobilized Peripheral Blood 14-Day CFU Analysis Package	22007

#### System is supplied with:

- STEMvision™ base unit (#22102C)
- Computer and monitor (#22101)
- Software for image acquisition, analysis and review (Catalog #22008, #22009 and/or #22011 as selected)
- One- or two-year warranty

#### Required reagents:

- HetaSep™ or other method for RBC removal (page 12)
- MethoCult™ Express or MethoCult™ Optimum (page 13)
- SmartDish™ cultureware (page 14)

## References

- 1. Migliaccio AR, et al. Blood 96: 2717-2722, 2000
- 2. Iori AP, et al. Bone Marrow Transplantation 33: 1097-1105, 2004
- 3. Yoo KH, et al. Bone Marrow Transplantation 39: 515 521, 2007
- 4. Prasad VK, et al. Blood 112: 2979-2989, 2008
- 5. Page KM, et al. Biol Blood Marrow Transplant 17: 1362-1374, 2011

#### Capacity:

- One 6-well SmartDish™ at a time
- Imaging each individual well of a 6-well SmartDish™ takes approximately 1 minute
- Image analysis takes approximately 1 minute/well but can be performed at a later time

#### **Dimensions:**

- 478 mm W x 335 mm D x 347 mm H
- 18.82 in W x 13.19 in D x 13.66 in H

#### Weight:

- STEMvision™: 59 lbs or 27 kg
- Computer: 28 lbs or 12 kg

#### **Power Requirements:**

- 100 240 V~, 50/60 Hz, 1.6 A
- Fuse 250V 2A Fast Blow

#### **Optimal Operating Conditions:**

- 15 30°C
- 20 85% relative humidity
- Not specified for use inside an incubator
- Does not require placement in a biohazard safety cabinet
- Indoor use only
- Not to be used in a cold room

#### **Storage Conditions:**

- -30°C to 50°C
- 10 90% relative humidity

For related products for HSPC research, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit **www.stemcell.com/HSPCworkflow** or contact us at **techsupport@stemcell.com**. For available fresh and cryopreserved peripheral blood, cord blood and bone marrow products in your region, visit **www.stemcell.com/primarycells**.



# CFU ASSAYS OF HUMAN HEMATOPOIETIC CELLS

Automated and Standardized Counting with STEMvision<sup>™</sup>



TOLL FREE PHONE 1 800 667 0322
PHONE +1 604 877 0713
INFO@STEMCELL.COM
TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT WWW.STEMCELL.COM