

CFU ASSAYS FOR CORD BLOOD BANKS

Automated and Standardized
Counting with STEMvision™



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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.

The Colony-Forming Unit (CFU) Assay

for Cord Blood Banks



The colony-forming unit (CFU) assay is the gold standard in vitro functional assay for identifying and counting hematopoietic progenitor cells. It can be used by cord blood (CB) banks to evaluate the viability and CFU content of cell products.

The utility of the CFU assay for CB stem cell transplantation has been demonstrated by numerous clinical studies involving patients with a variety of hematological and non-hematological cancers and other disorders. These studies show that the number of CFUs in a CB unit, especially in cryopreserved cells thawed for unrelated transplantation, is the one parameter that best correlates with the time to neutrophil and platelet engraftment, and overall survival following CB transplantation.¹⁻⁵

Based on these studies, several regulatory bodies (e.g. FDA, AABB, NetCord-FACT) recommend or require that the number of CFUs in a CB unit be measured before cryopreservation and/or after thawing, prior to release of the unit to a transplant facility. STEMCELL Technologies offers a complete set of tools for CB banks to determine the number of CFUs in CB samples (Figure 1).

Public Cord Blood Banks

The CFU assay can be used to identify CB units containing a high number of progenitor cells. Pre-selection of CB units with a high CFU content ensures that valuable space and resources are expended only on banking the most promising units, and thereby improves the quality of the CB repository.

The CFU assay can also be used to determine whether processing and cryopreservation have adversely affected the number of viable progenitor cells in a CB unit. This ensures that the procedures used in individual laboratories yield CB products with high hematopoietic potential.

Finally, the CFU assay can be used to assist in selecting one or more suitable CB unit(s) for transplantation. The number of viable and functional CFUs in a CB unit after thawing is a key determinant guiding selection of units with high hematopoietic potential.

Private Cord Blood Banks

The CFU assay provides families with important biological information about the quality of their child's CB sample at the time of collection. It can provide parents with assurance that the hematopoietic progenitor cells in their child's CB are viable in the event that the unit is needed for hematopoietic rescue later in life. This information can also help parents make their financial investment with greater confidence.

The benefits of the CFU assay can be highlighted to families in the process of deciding which bank to use to process and store their CB cells. It offers a means for private CB banks to differentiate themselves from their many competitors in the marketplace.

A Complete Workflow for Cord Blood CFU Assays:

- HetaSep™ for removing red blood cells from fresh CB samples
- MethoCult™ Express and MethoCult™ Optimum media for 7- and 14-day CFU assays, respectively
- SmartDish™ meniscus-free cultureware for more accurate CFU counting
- STEMvision™ for automated and standardized counting of BFU-E, CFU-G/M/GM and CFU-GEMM
- Analysis Packages for counting 7-day and 14-day CB CFU assays
- Proficiency testing programs

The CFU Assay Workflow

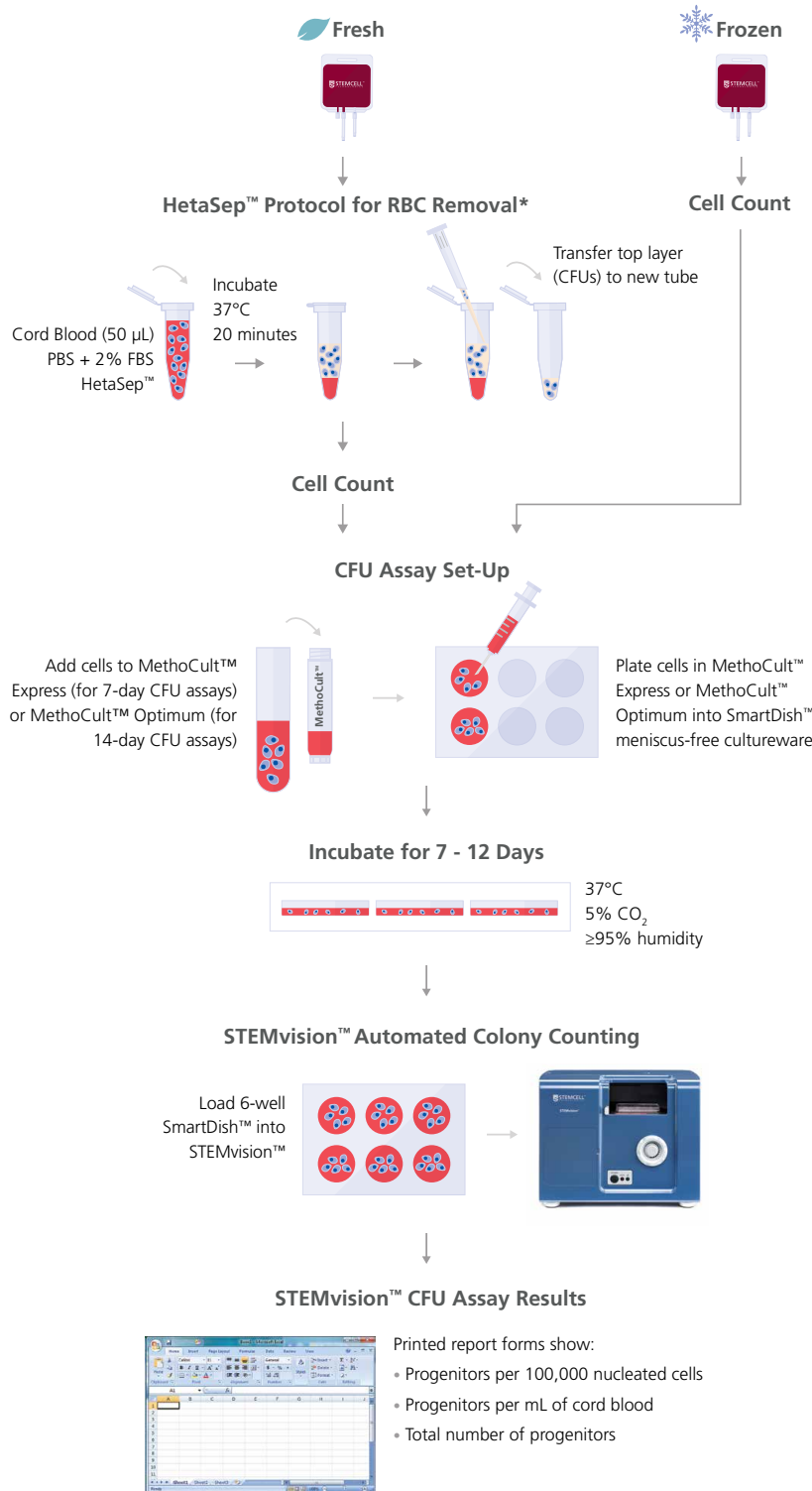


Figure 1. A Typical CFU Assay Workflow for a CB Bank With STEMvision™ Integrated for Automated Counting of Hematopoietic Colonies

Red blood cells (RBCs) are removed from a 50 mL sample of fresh cord blood (CB) using HetaSep™. This step is not required for thawed cryopreserved CB cells. Fresh or thawed CB cells are then cultured in the appropriate MethoCult™ medium, depending on whether the different colony-forming unit (CFU) subtypes will be scored after 14-days (MethoCult™ Optimum) or if total CFU numbers will be counted after 7-days (MethoCult™ Express). STEMvision™ is used to acquire an image of the culture dish and analyze the image to identify and enumerate the different colony types. The results of the CFU assay can be documented in the form of a printed report showing the frequency and total number of CFUs in the CB unit. These forms are available in two formats; one for the lab and another 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.

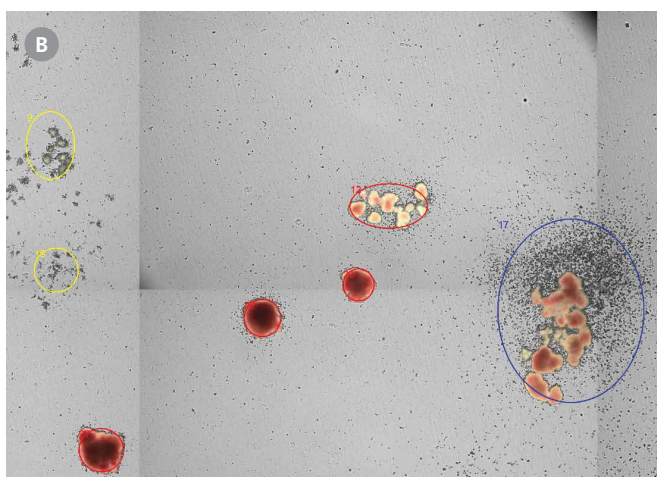
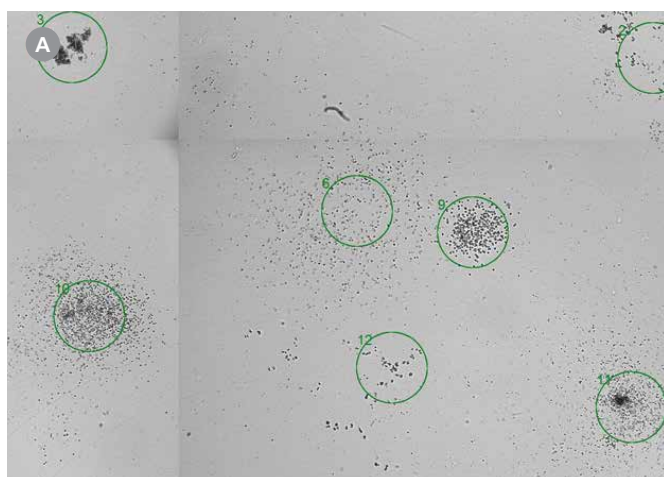


Figure 2. Representative STEMvision™ Images Showing Colonies Derived from CB Progenitors After 7 Days of Culture in MethoCult™ Express or 14 Days of Culture in MethoCult™ Optimum

The images have been analyzed with the STEMvision™ Human CB (A) 7-Day and (B) 14-Day 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-G, CFU-M or CFU-GM) 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.

STEMvision™ Can Help You:

- Automate and standardize colony counting to ensure accurate and reproducible CFU assay results
- Minimize intra- and inter-individual and laboratory variation in colony counting
- Digitally image cultures for permanent record keeping
- Collect and review data in an easy to use format
- Save time in staff training and laboratory workflows

STEMvision™ Performance Data



The total number of hematopoietic colonies and the numbers of erythroid, myeloid and mixed colony sub-types in colony-forming unit (CFU) assays of cord blood (CB) cells counted using STEMvision™ is highly correlated with the number of colonies counted manually using an inverted microscope. Importantly, colony counts produced by STEMvision™ show significantly lower variability in the recommended range of 20-80 colonies per 35 mm culture well than colony counts produced manually by multiple technicians scoring the same CFU assays.

The correlation between automated and manual colony counting, and the reduced variability observed with automated CFU counting are shown in Figures 3-5.

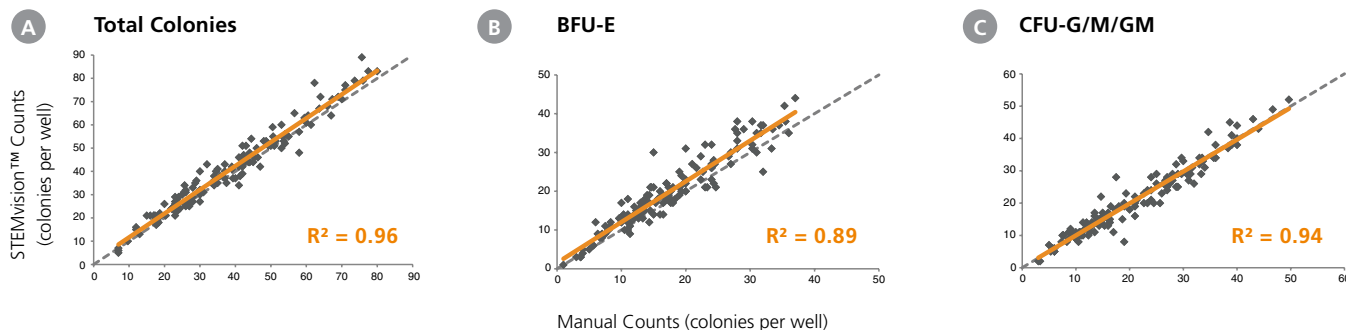


Figure 3. STEMvision™ Automated Counts of Total, Erythroid (BFU-E) and Myeloid (CFU-G/M/GM) Colonies are Highly Correlated to Manual Counts of 14-Day CB CFU Assays

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^2) that describe each data set ($n=130$ CFU assays) are as follows:

- Figure 3A: $y=1.02x + 1.39$; $R^2=0.96$ for Total Colonies
- Figure 3B: $y=1.05x + 1.53$; $R^2=0.89$ for BFU-E
- Figure 3C: $y=0.99x + 0.13$; $R^2=0.94$ for CFU-G/M/GM

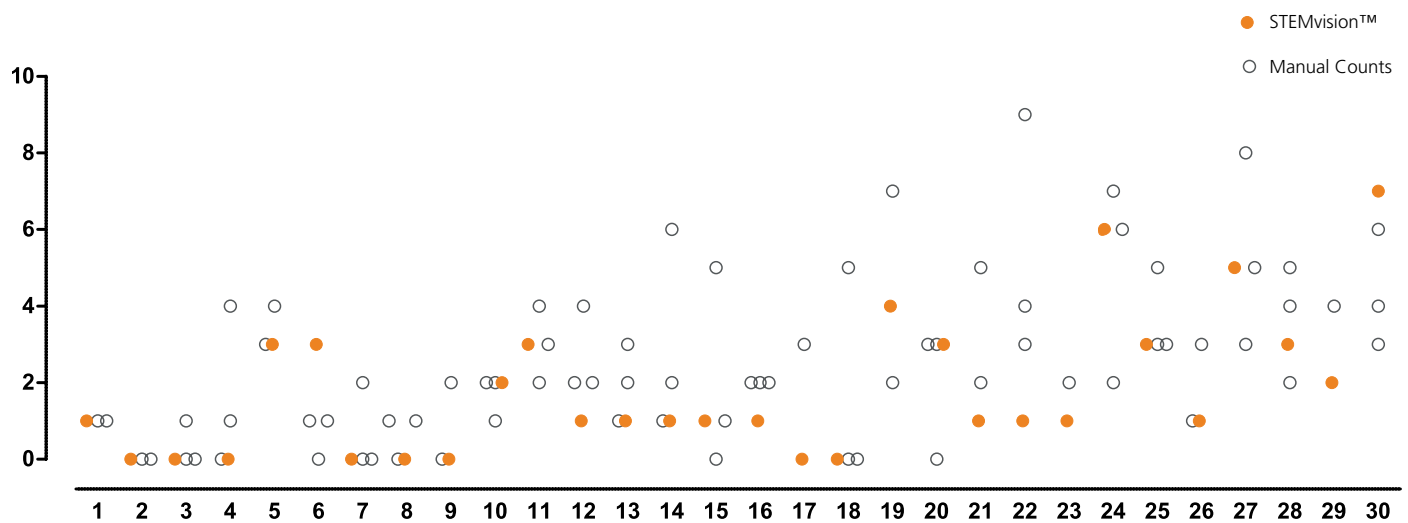


Figure 4. STEMvision™ Automated Counting of Mixed Colonies Falls Within the Range of Manual Counts of 14-Day CB CFU Assays

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.

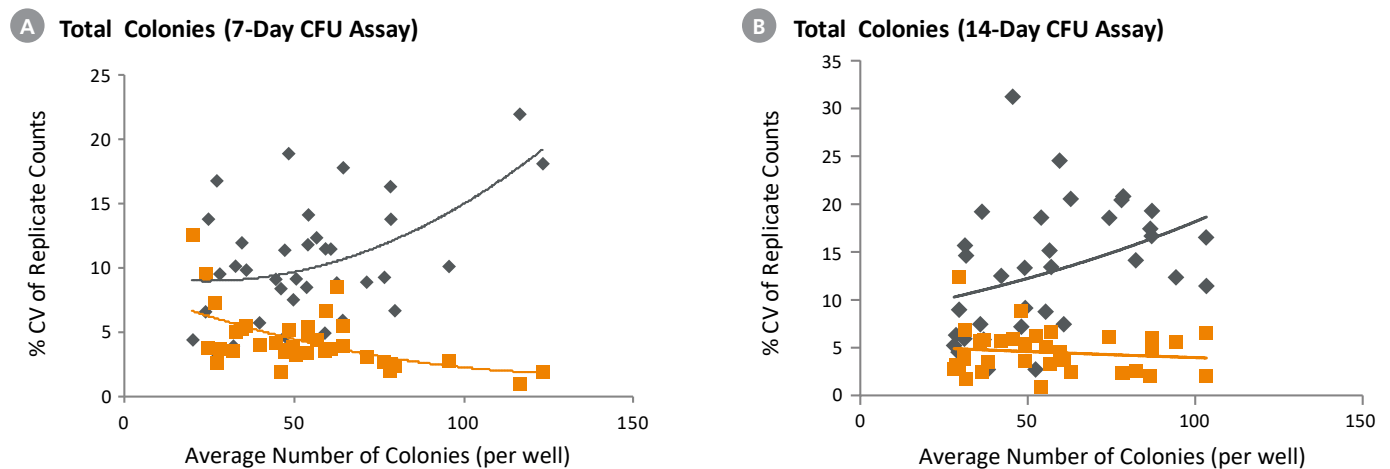


Figure 5. STEMvision™ Automated Colony Counting of 7-Day and 14-Day CB CFU Assays is More Reproducible Than Manual Counting

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 cord blood (CB) unit and the colony-forming unit (CFU) assay results (Figure 6). These reports provide critical functional information about the CB sample for the bank's own records and for the family (if desired). The values documented in these reports include:

- CB bank address and contact information
- Patient and doctor demographic information
- CB sample and CFU assay tracking ID numbers
- Number of viable progenitor cells per 100,000 nucleated cells
- Number of viable progenitor cells per mL of CB
- Total number of viable progenitor cells in the CB unit
- Counts for 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)

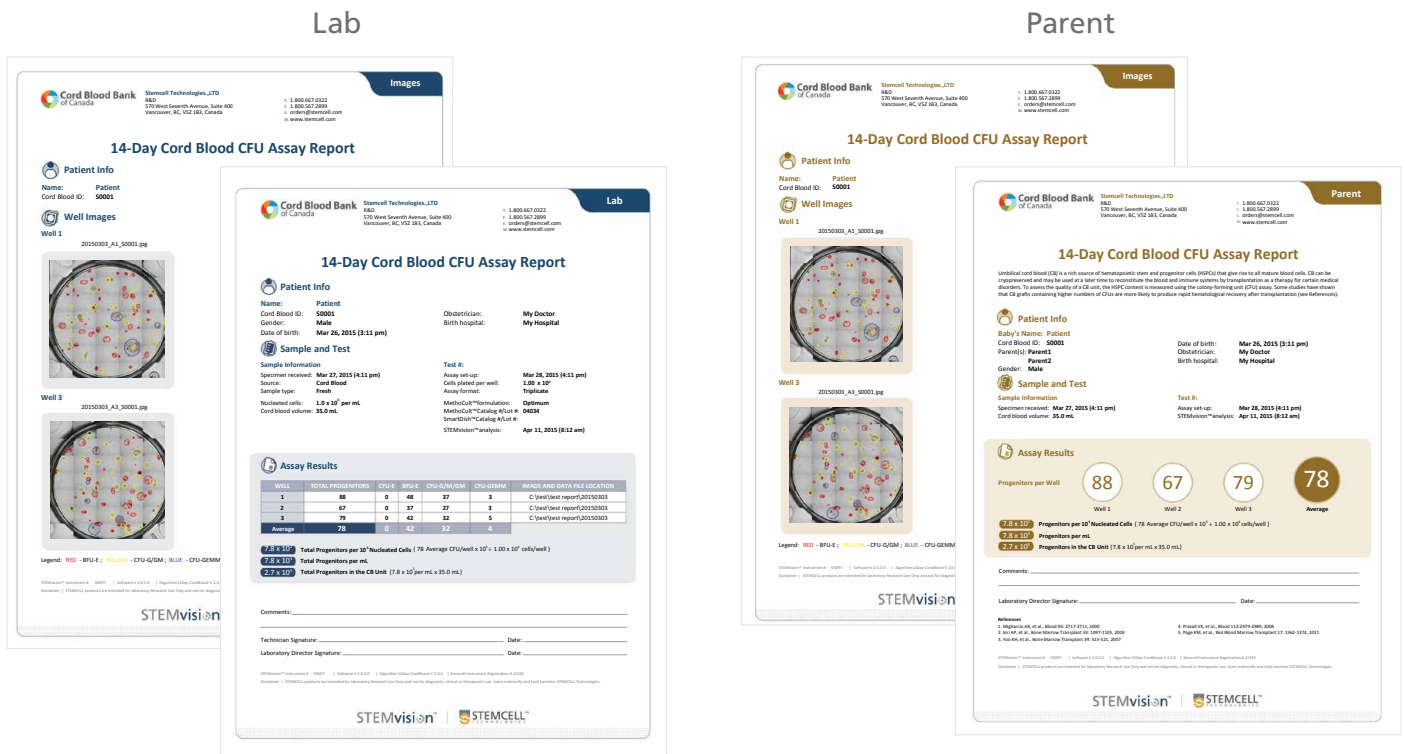


Figure 6. Sample STEMvision™ Lab and Parent Reports for a 14-Day Cord Blood CFU Assay

Printed reports can be generated in two versions, as both (A) Lab, for the CB bank's own records, and (B) Parent, for the family, if desired.

ErythroClear™

Depletion of Red Blood Cells from Small Volume Cord Blood Samples



ErythroClear™ Red Blood Cell Depletion Reagent and Magnet

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.

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.

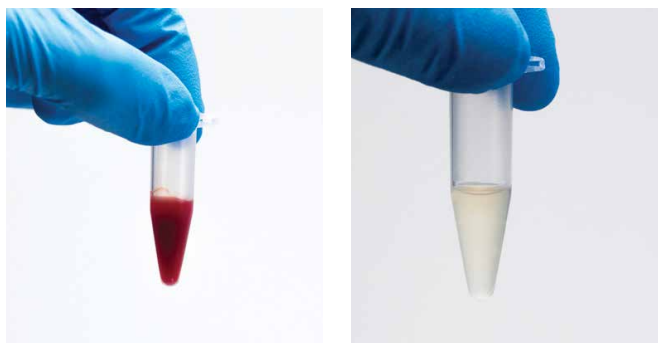
Why Use ErythroClear™ ?

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.



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

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.

HetaSep™

Depletion of Red Blood Cells from Fresh Blood Samples



HetaSep™

Product	Quantity	Catalog #
HetaSep™ (6-Well Plates)	20 mL	07806
	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.

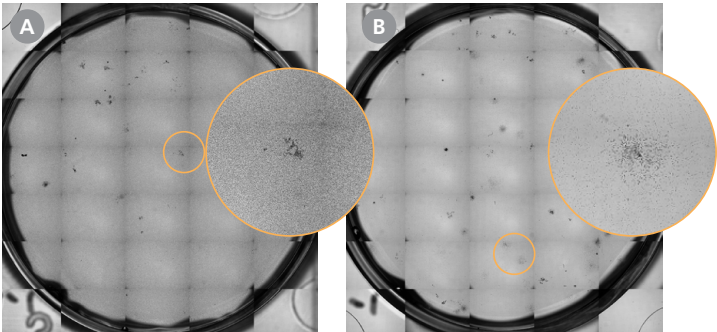


Figure 7. STEMvision™ Images of 7-Day CFU Assays of Fresh Cord Blood Samples Plated in MethoCult™ Express without and with Prior Removal of RBCs Using HetaSep™

(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.

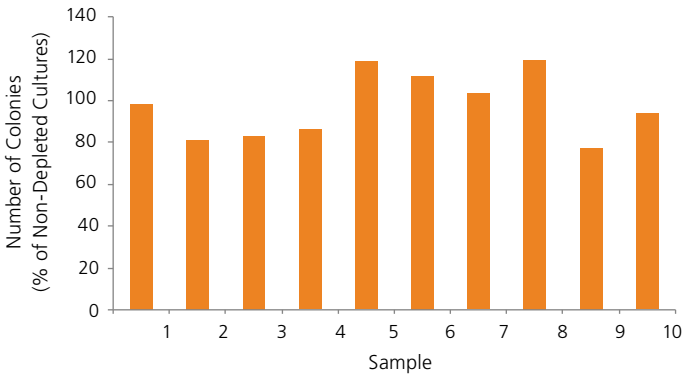


Figure 8. An Average of 97% of Colony-Forming Cells are Recovered Following RBC Depletion with HetaSep™

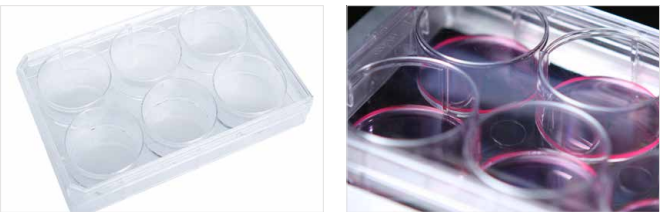
Cord blood samples (n = 10) were split into two parts, one of which was plated in a CFU assay without RBC depletion and the other having RBCs depleted using the HetaSep™ protocol before plating. Each sample type was plated in duplicate. CFU assays were counted manually and the percent recovery of colonies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

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™ plates may be counted using automated methods.

SmartDish™ Meniscus-Free Cultureware

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

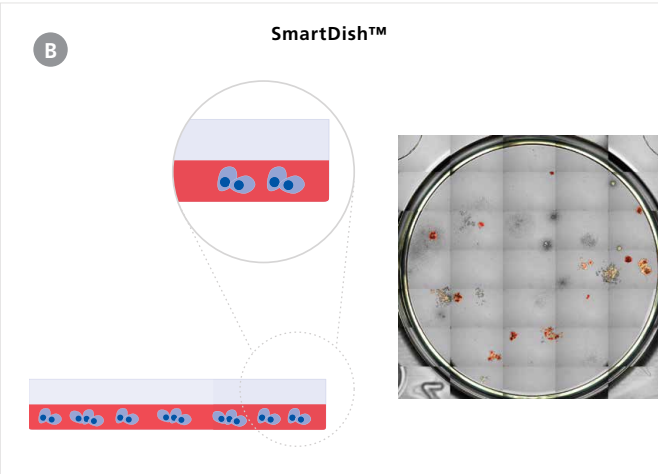
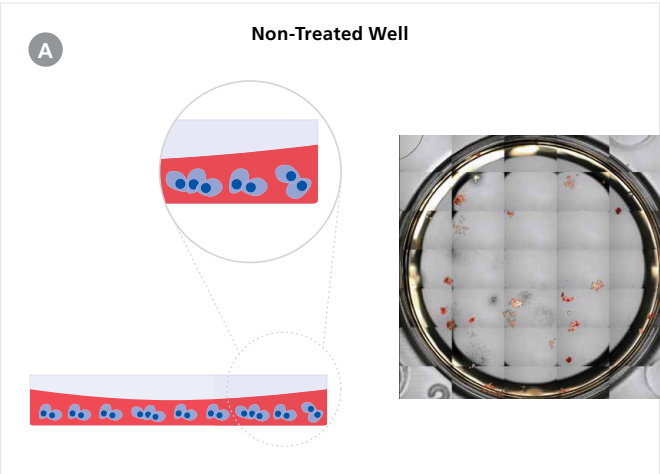


Figure 9. 14-Day CB CFU Assays Performed in Standard Non-Treated and SmartDish™ 6-Well Culture Plates.

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 standard cultureware causes more colonies to form around the periphery of the dish where the culture medium is deeper (A). 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).

MethoCult™ Optimum

Methylcellulose Medium for 14-Day CFU Assays

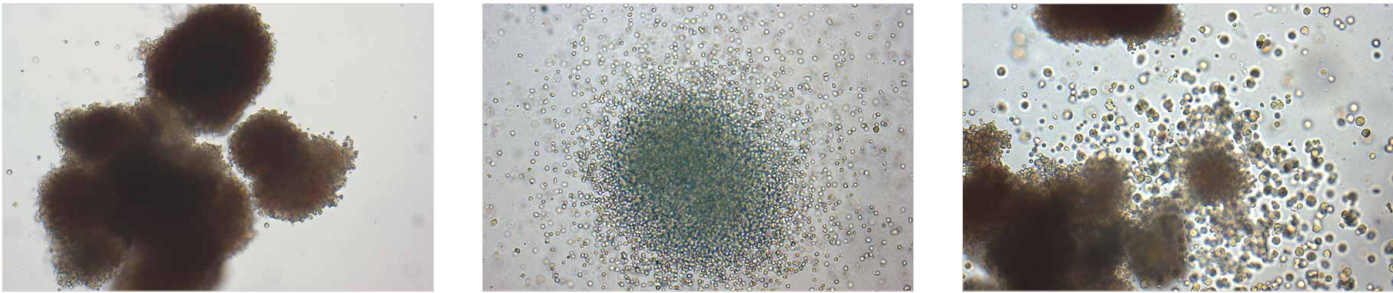


Figure 10. Microscope Images of Colonies Derived From BFU-E, CFU-GM and CFU-GEMM Progenitors, Respectively, After 14-Day Cultures in MethoCult™ Optimum

The STEMvision™ Human Cord Blood 14-Day Analysis Package has been designed for use with MethoCult™ Optimum medium (Catalog #04034/04044). This medium is considered to be the gold standard in the hematopoiesis field and is used extensively in global proficiency testing programs. MethoCult™ Optimum is formulated to support optimal proliferation and differentiation of BFU-E, CFU-G/M/GM and CFU-GEMM.

For more details, please visit www.stemcell.com/CFUwallchart.

MethoCult™ Optimum is CE Marked for in vitro diagnostic (IVD) use in the European Union (EU). The CE Mark indicates that this medium complies with EU safety, environmental and quality standards required for IVD medical devices. It complies with Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Diagnostic Medical Devices within applicable countries.

Outside of the EU, MethoCult™ Optimum is for research use only unless otherwise determined, not for therapeutic or diagnostic use.

Benefits of MethoCult™ Optimum:

- Formulated for use with cord blood, isolated CD34⁺ cells and other tissues
- Optimized for a wide range of applications
- Available in formulations with and without EPO



TECHNICAL BULLETIN

"Potency" Assays for Measuring the Engraftment Potential of Hematopoietic Stem and Progenitor Cells

www.stemcell.com/potency_assay

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

MethoCult™ Product	Catalog #	Size	Components				Applications
			MC	FBS	BSA	Growth Factors	
MethoCult™ Optimum	04034/84434* 04044/84444*	100 mL 24 x 3 mL	✓	✓	✓	Cytokines, including erythropoietin (EPO)	Supports growth of CFU-E, BFU-E, CFU-G/M/GM and CFU-GEMM in human CB

MC: methylcellulose; FBS: fetal bovine serum; BSA: bovine serum albumin; CB: cord blood

*CE Marked for IVD use in the EU.

**Please contact Tech Support for more information.

MethoCult™ Express

Methylcellulose Medium for 7-Day CFU Assays

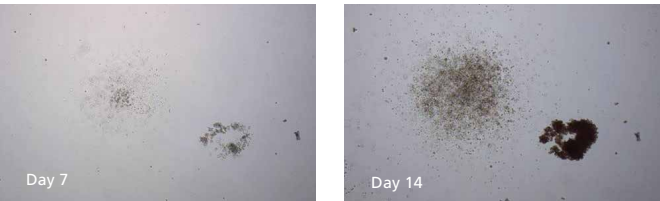


Figure 11. Hematopoietic Colonies Grown in MethoCult™ Express, Visualized on Day 7 (Left) and Day 14 (Right) Under a Microscope

Benefits of MethoCult™ Express:

- Formulated for accelerated progenitor cell proliferation and colony formation
- Allows counting of total CFU numbers from CB samples in only 7 days

Historically, human colony-forming unit (CFU) assays have been counted after 14 days. This period allows sufficient time for progenitor cells to differentiate into the different hematopoietic lineages so that the various sub-types of CFUs can be scored separately. While these 14-day assays are preferred by many labs, they are not essential to simply measure the total number of viable and functional CFUs.

The STEMvision™ Human Cord Blood 7-Day Analysis Package is designed to be used with MethoCult™ Express (Catalog #04437/04447). This medium is formulated to accelerate the proliferation of hematopoietic progenitor cells so that colonies can be counted after only 7 days; one week faster than with a conventional 14-day CFU assay. This approach provides a simple method to more quickly determine the total number of viable and functional progenitor cells in a cord blood (CB) unit. The total number of CFUs in a CB unit has been shown by several clinical studies to strongly correlate with engraftment outcomes following CB transplantation.¹⁻⁵

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 (Catalog #04034/04044), or in other formulations, including MethoCult™ Classic (Catalog #04434/04444) and MethoCult™ Enriched (Catalog #04435/04445).

MethoCult™ Express is CE Marked for in vitro diagnostic (IVD) use in the European Union (EU). The CE Mark indicates that this medium complies with EU safety, environmental and quality standards required for IVD medical devices. It complies with Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on In Vitro Diagnostic Medical Devices within applicable countries.

Outside of the EU, MethoCult™ Express is for research use only unless otherwise determined, not for therapeutic or diagnostic use.

Table 2. MethoCult™ Express Media Currently Validated for Automated Counting with STEMvision™**

MethoCult™ Product	Catalog #	Size	Components				Applications
			MC	FBS	BSA	Growth Factors	
MethoCult™ Express	04437* 04447*	100 mL 24 x 3 mL	✓	✓	✓	Cytokines, including erythropoietin (EPO)	Measures the total number of CFUs in cord blood in only 7 days

MC: methylcellulose; FBS: fetal bovine serum; BSA: bovine serum albumin
*CE Marked for IVD use in the EU.
**Please contact Tech Support for more information.

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

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™
- MethoCult™ GF M3434, GF M3534 and/or SF M3436
- SmartDish™ cultureware

Capacity:

- One 6-well SmartDish™ at a time
- Image acquisition takes approximately 30 seconds/well
- Image analysis takes approximately 30 seconds/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 to be used inside a cold room or 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, including specialized culture media and supplements, storage media and antibodies visit www.stemcell.com/CBworkflow or contact us at techsupport@stemcell.com.

References

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3. Yoo KH, et al. Bone Marrow Transplantation 39: 515-521, 2007
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