

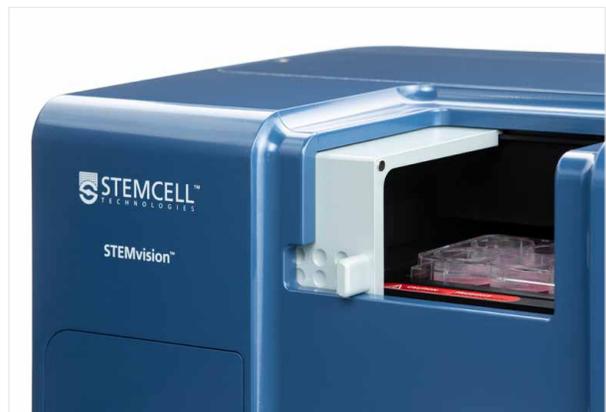
MOUSE BONE MARROW CFU ASSAYS

Automated and Standardized
Counting with STEMvision™



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STEMCELL Technologies, Inc. offers a comprehensive line of products for laboratories performing basic research on mouse hematopoietic stem and progenitor cells. STEMCELL Technologies' Quality Management System is certified to ISO 13485 Medical Device Standards.

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

Colony-Forming Unit (CFU) Assays

for Mouse Bone Marrow

Mouse models are important tools for scientists investigating the biology and genetics of hematopoietic stem and progenitor cells (HSPCs) *in vivo*. The study of mouse HSPCs has allowed us to better understand the origin of human hematopoietic stem cells (HSCs) in development, to develop functional assays for their identification, and to identify the key regulators controlling their self-renewal and differentiation.

HSCs are identified by their ability to regenerate and maintain long-term multilineage hematopoiesis after transplantation. More mature progenitor cells, with restricted developmental potential, are typically identified using *in vitro* assays. The colony-forming unit (CFU) assay is the gold standard *in vitro* functional assay for measuring the number of progenitor cells in mouse hematopoietic cell populations and has numerous applications for basic research in hematopoiesis.

Historically, the CFU assay has been performed by culturing hematopoietic cells in MethoCult™ medium and counting the number of colonies produced by different subtypes of CFUs 7 to 12 days later using an inverted microscope. Lineage-restricted (i.e. colony-forming unit – erythroid (CFU-E), burst-forming unit – erythroid (BFU-E), colony-forming unit – granulocyte (CFU-G), colony-forming unit – macrophage (CFU-M), colony-forming unit – granulocyte, macrophage (CFU-GM)) and multi-potential progenitor cells (i.e. colony-forming unit – granulocyte, erythrocyte, macrophage and megakaryocyte (CFU-GEMM)), are stimulated to proliferate by specific combinations of cytokines and the colonies they produce are then identified and counted.

Accurate counting of colonies can be challenging for individuals with limited experience. It takes time to train new students and technicians to properly recognize and count hematopoietic colonies, and the process of counting is itself time consuming for laboratories that perform and analyze large numbers of CFU assays each day.

To save time and standardize colony counting for mouse bone marrow (BM) CFU assays, investigators can use STEMvision™, a bench-top instrument and computer system designed specifically for automated imaging and counting of hematopoietic colonies in the CFU assay. This system is optimized for use with MethoCult™ media and meniscus-free SmartDish™ cultureware (Figure 1). The use of this standardized platform significantly improves the accuracy and reproducibility of mouse CFU assay counting and analysis.

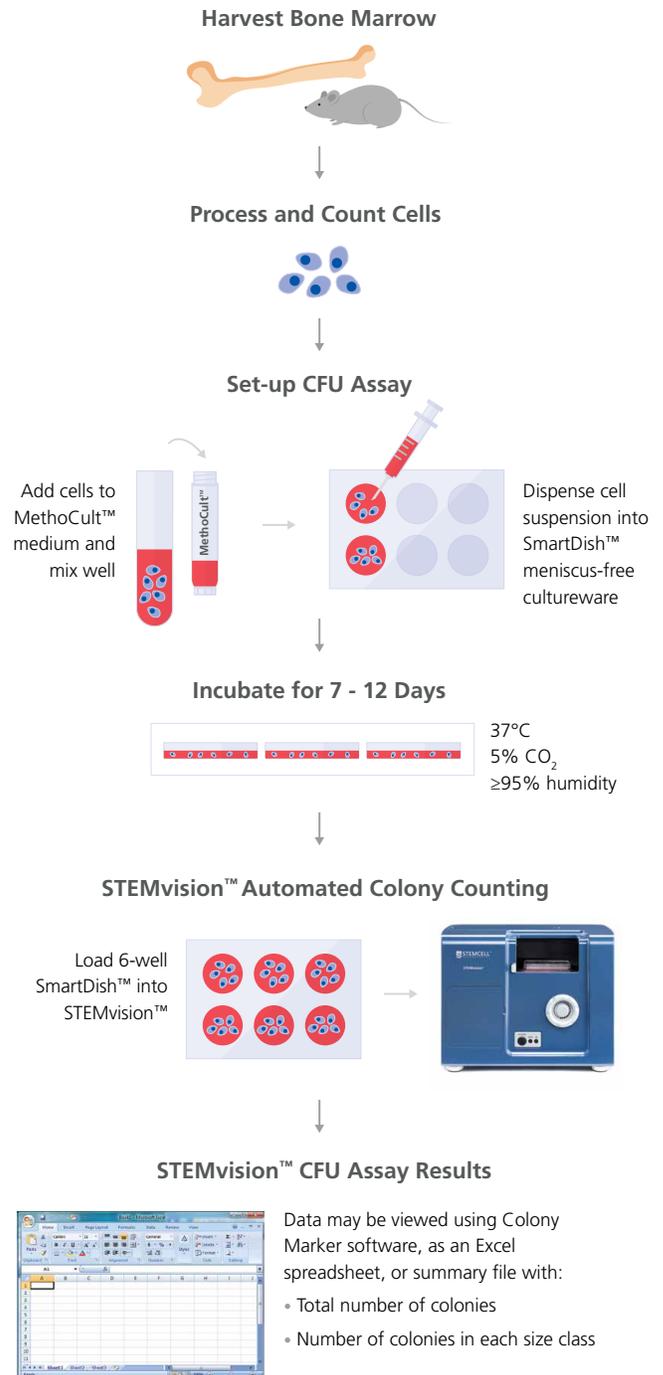


Figure 1. A Typical CFU Assay Workflow Incorporating STEMvision™ for Automated Counting of Mouse BM Colonies

Bone marrow (BM) cells are harvested from a mouse, processed and counted. Cells are diluted and added to MethoCult™ medium at the appropriate concentration and mixed well. MethoCult™ medium is then dispensed into single wells of a 6-well SmartDish™ plate. After incubation for 7 - 12 days, STEMvision™ is used to acquire an image of each culture well. The total number of colonies is counted using the appropriate software Analysis Package (Table 1). Colonies are also categorized based on their size. Results may be viewed in spreadsheet or summary formats.

STEMvision™

Automated CFU Assay Imaging and Standardized Colony Counting

STEMvision™ replaces the need to manually count mouse bone marrow (BM) colonies with a microscope. To automate the process of BM colony counting, simply load a SmartDish™ culture plate containing up to 6 CFU assays into STEMvision™. The instrument captures an image of each 35 mm well in approximately 30 seconds. Highly sophisticated image analysis software is then used to count each colony in approximately 30 seconds, classifying them into one of four size classes that users can choose to include or exclude from the final count of total colonies (Figure 2).

Culture wells can be imaged and analyzed in a single consecutive step, or for high-throughput processing, multiple dishes can be imaged sequentially and then analyzed overnight or at a later time.

Software (Analysis Packages) for CFU Counting

Software for STEMvision™, called Analysis Packages, have been developed to accurately count the total number of colonies in CFU assays of mouse BM cells plated in MethoCult™ media and analyzed after 7 to 12 days (Table 1). These Analysis Packages produce total counts of (i) combined myeloid and erythroid progenitor cells, (ii) myeloid progenitor cells only or (iii) erythroid progenitor cells only, without differentiating between progenitor subtypes (e.g. CFU-G vs CFU-M).

Analysis Packages are designed to pair with the MethoCult™ medium that is specific for your progenitor cells of interest:

- For assays using MethoCult™ GF M3434, the Mouse Total CFU Analysis Package (Catalog #22008) provides a total count of combined myeloid plus erythroid colonies (Figure 3).



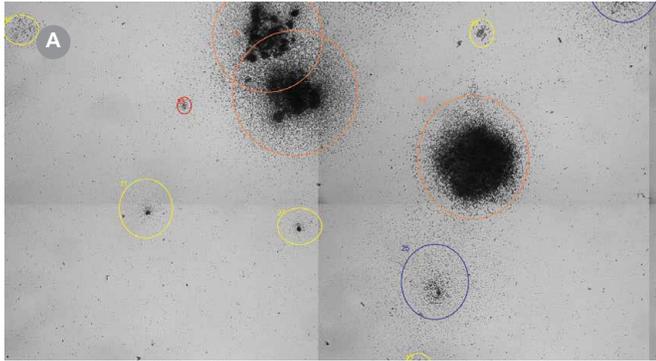
- For assays using myeloid-specific MethoCult™ GF M3534, the Mouse Myeloid CFU Analysis Package (Catalog #22009) provides a total count of all myeloid colonies (Figure 4).
- For assays using erythroid-specific MethoCult™ SF M3436, the Mouse Erythroid CFU Analysis Package (Catalog #22011) provides a total count of all erythroid colonies (Figure 5).
- For differential counts of myeloid and erythroid progenitors in a single sample, cells must be cultured in MethoCult™ GF M3534 and MethoCult™ SF M3436 in parallel. For investigators interested in this option, we provide bundles containing both the Myeloid CFU and Erythroid CFU Analysis Packages (Catalog #22012) together, and both MethoCult™ GF M3534 and MethoCult™ SF M3436 formulations (Catalog #22013).

Finally, STEMvision™ will subdivide colony counts into four size classes (class 1 being the smallest to class 4, the largest) based on colony size and density. This feature allows researchers to define size cutoffs so that very small colonies can be excluded from total colony counts if desired.

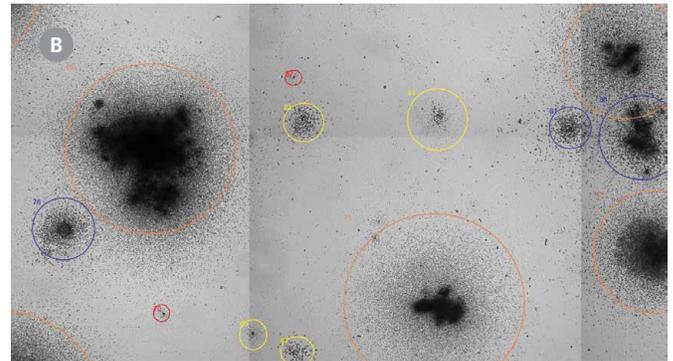
Advantages of STEMvision™:

- More accurate counting of colonies in media that support the growth of myeloid and/or erythroid progenitors
- Reduces inter- and intra-laboratory variation in colony counting
- Saves time in staff and student training
- Produces digital images of cultures for permanent record keeping
- Results presented in spreadsheet and summary formats

MethoCult™ GF M3434



MethoCult™ GF M3534



MethoCult™ SF M3436

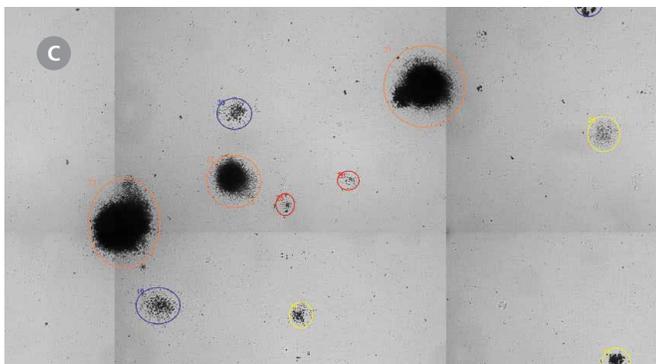


Figure 2. Representative STEMvision™ Images Showing Colonies Derived from Mouse BM Progenitors After 12 Days of Culture in MethoCult™ GF M3434, MethoCult™ GF M3534 or MethoCult™ SF M3436 Media

Images of mouse BM cells cultured in (A) MethoCult™ GF M3434, (B) MethoCult™ GF M3534 and (C) MethoCult™ SF M3436 were acquired using STEMvision™. The corresponding STEMvision™ Mouse Analysis Package (Table 1) was used to analyze each 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.

Table 1. MethoCult™ Media and Software for Automated Colony Counting With STEMvision™

MethoCult™ Medium	Growth Factors	Corresponding Analysis Package	Application
MethoCult™ GF M3434 (Catalog #03434/03444)	SCF, IL-3, IL-6, EPO	Mouse Total CFU Analysis Package (Catalog #22008)	Total count of combined myeloid plus erythroid progenitors (CFU-GEMM, BFU-E, CFU-G/M/GM) in mouse BM*
MethoCult™ GF M3534 (Catalog #03534)	SCF, IL-3, IL-6	Mouse Myeloid CFU Analysis Package (Catalog #22009)	Total count of myeloid progenitors only (CFU-G/M/GM) in mouse BM*
MethoCult™ SF M3436 (Catalog #03436)	Cytokines, including EPO	Mouse Erythroid CFU Analysis Package (Catalog #22011)	Total count of erythroid progenitors only (BFU-E) in mouse BM*

BM: Bone Marrow; SCF: Stem Cell Factor; EPO: Erythropoietin; BFU-E: Burst-Forming Unit - Erythroid; CFU-G: Colony-Forming Unit - Granulocyte; CFU-M: Colony-Forming Unit - Macrophage; CFU-GM: Colony-Forming Unit - Granulocyte/Macrophage; CFU-GEMM: Colony-Forming Unit - Granulocyte, Erythrocyte, Macrophage, Megakaryocyte.

*Isolated from C57BL/6 mice

STEMvision™ Performance Data

Automated Counting of Mouse Bone Marrow CFU Assays on Days 7, 10 and 12

MethoCult™ GF M3434: Myeloid and Erythroid Colonies

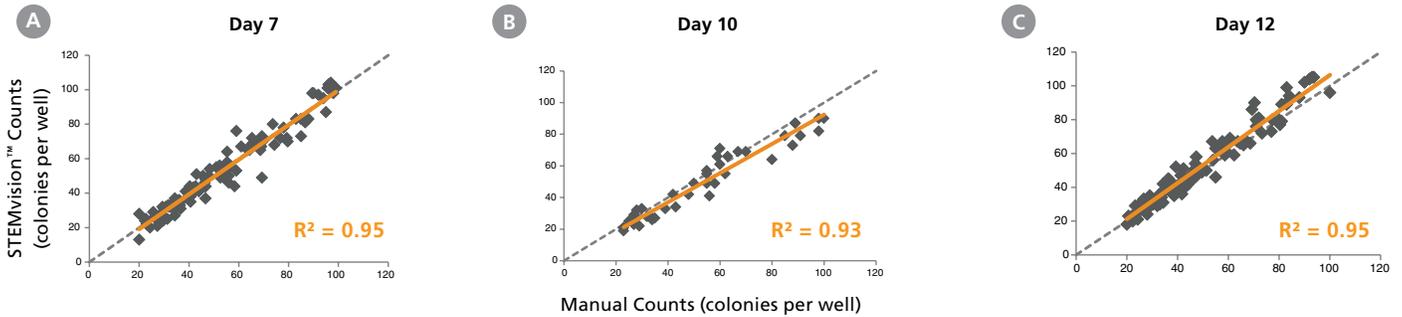


Figure 3. STEMvision™ Automated Counting is Highly Correlated to Manual Counting of Total (Myeloid Plus Erythroid) Colonies in Mouse BM CFU Assays

BM cells were plated in MethoCult™ GF M3434 (Catalog #03434/03444). 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. The slope, 95% confidence intervals, coefficients of determination (R²) and sample size for each data set are provided in Table 2.

MethoCult™ GF M3534: Myeloid Colonies Only

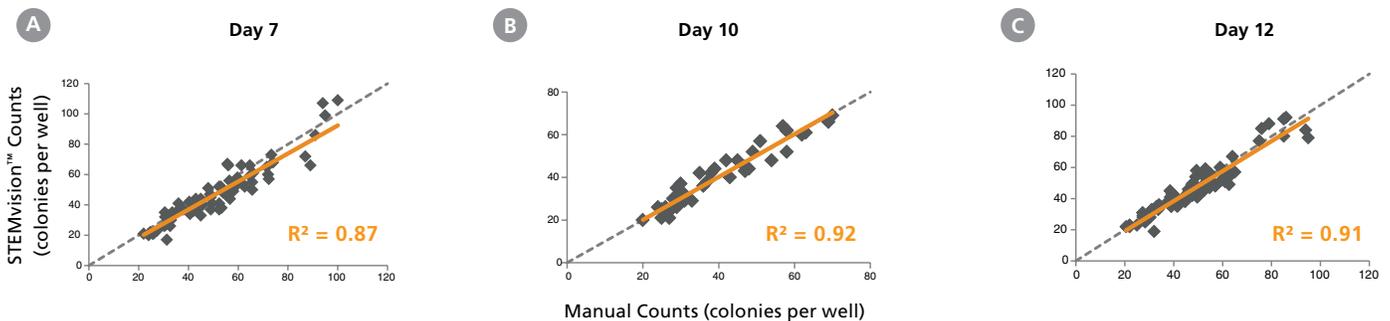


Figure 4. STEMvision™ Automated Counting is Highly Correlated to Manual Counting of Myeloid Colonies in Mouse BM CFU Assays

BM cells were plated in MethoCult™ GF M3534 (Catalog #03534). 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 Myeloid CFU Analysis Package (Catalog #22009). We recommend counting CFU assays of mouse myeloid progenitor cells plated in M3534 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. The slope, 95% confidence intervals, coefficients of determination (R²) and sample size for each data set are provided in Table 2.

MethoCult™ SF M3436: Erythroid Colonies Only

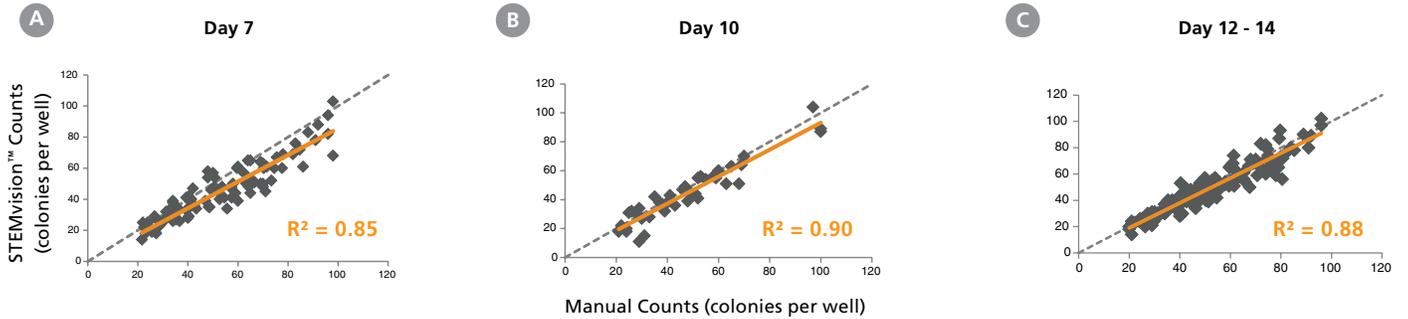


Figure 5. STEMvision™ Automated Counting is Highly Correlated to Manual Counting of Erythroid Colonies in Mouse BM CFU Assays

BM cells were plated in MethoCult™ SF M3436 (Catalog #03436). Colonies were counted on days (A) 7, (B) 10 and (C) 12 - 14 both manually using an inverted microscope, and automatically using STEMvision™ equipped with the Mouse Erythroid CFU Analysis Package (Catalog #22011). We recommend counting CFU assays of mouse erythroid progenitor cells plated in M3436 between of 10 to 14 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. The slope, 95% confidence intervals, coefficients of determination (R²) and sample size for each data set are provided in Table 2.

Table 2. Correlation Between Automated STEMvision™ and Manual Colony Counting

Methocult™ Medium (Colonies Supported)	Day Counted	Regression Analysis of STEMvision™ vs Manual Counts		Number of CFU Assays
		Slope, 95% CI*	Coefficient of Determination (R ²)	
MethoCult™ GF M3434 (Myeloid plus erythroid colonies)	Day 7	0.99 (0.97 - 1.01)	0.95	104
	Day 10	0.92 (0.89 - 0.96)	0.93	38
	Day 12	1.06 (1.05 - 1.08)	0.95	99
MethoCult™ GF M3534 (Myeloid colonies only)	Day 7	0.92 (0.89 - 0.95)	0.87	67
	Day 10	1.01 (0.98 - 1.04)	0.92	40
	Day 12	0.96 (0.94 - 0.99)	0.91	68
MethoCult™ SF M3436 (Erythroid colonies only)	Day 7	0.86 (0.83 - 0.88)	0.85	96
	Day 10	0.93 (0.89 - 0.97)	0.90	40
	Day 12 - 14**	0.95 (0.93 - 0.97)	0.88	131

*CI: Confidence Interval

**Mouse CFU assays of erythroid progenitor cells plated in M3436 should be counted between 10 to 14 days.



STEMvision™

Reduced Variability of Automated CFU Counts

An important advantage of STEMvision™ for automated and standardized counting of mouse CFU assays is that assay results are significantly more reproducible than with manual counting. The average coefficient of variation (CV) for replicate STEMvision™ colony counts, in the optimal range of 20 - 100 colonies per 35 mm culture well, is 4-fold lower than the average CV for manual colony counts produced by multiple people using a microscope.

The lower variability of STEMvision™ colony counts as compared to manual counts in CFU assays of total, myeloid and erythroid progenitors from mouse BM counted on day 12, is shown in Figure 6.

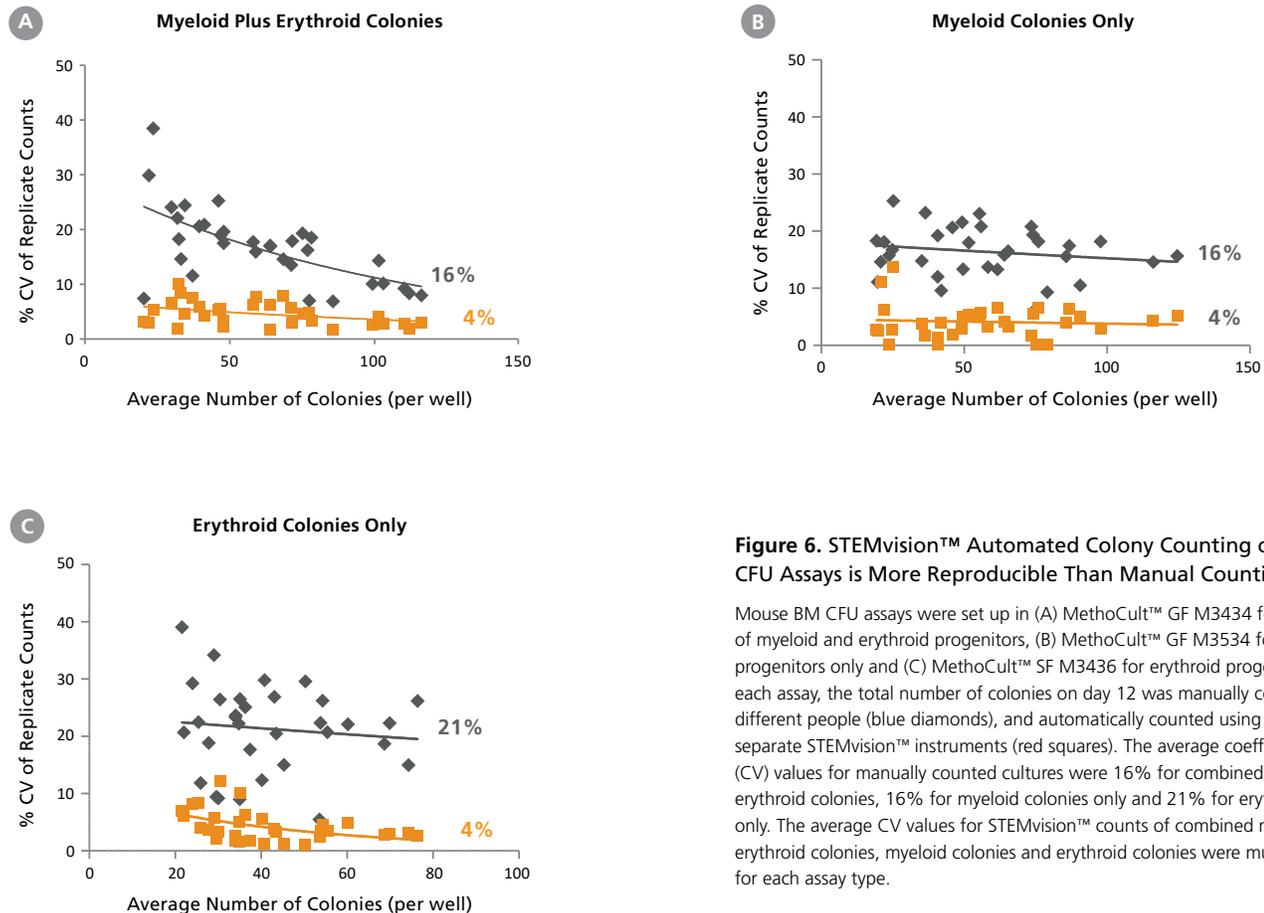
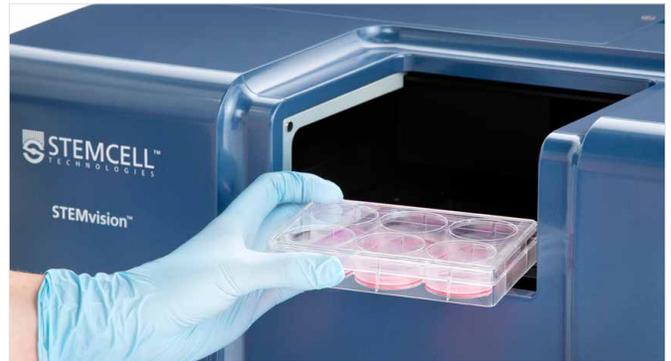


Figure 6. STEMvision™ Automated Colony Counting of Mouse BM CFU Assays is More Reproducible Than Manual Counting

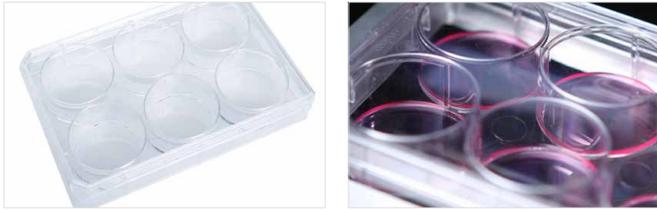
Mouse BM CFU assays were set up in (A) MethoCult™ GF M3434 for the growth of myeloid and erythroid progenitors, (B) MethoCult™ GF M3534 for myeloid progenitors only and (C) MethoCult™ SF M3436 for erythroid progenitors only. For each assay, the total number of colonies on day 12 was manually counted by five different people (blue diamonds), and automatically counted using three to four separate STEMvision™ instruments (red squares). The average coefficient of variation (CV) values for manually counted cultures were 16% for combined myeloid and erythroid colonies, 16% for myeloid colonies only and 21% for erythroid colonies only. The average CV values for STEMvision™ counts of combined myeloid and erythroid colonies, myeloid colonies and erythroid colonies were much lower at 4% for each assay type.

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

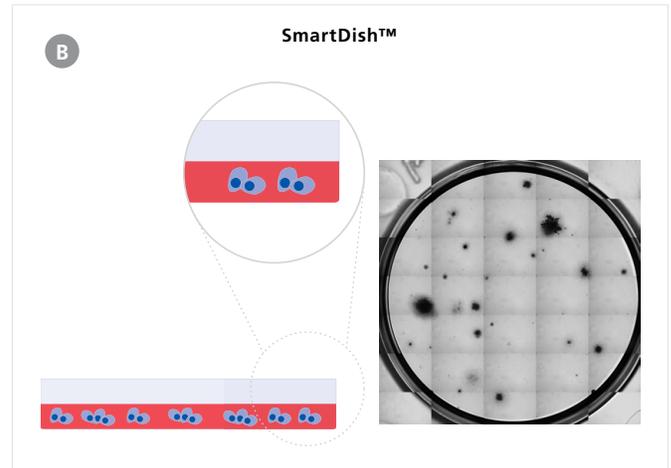
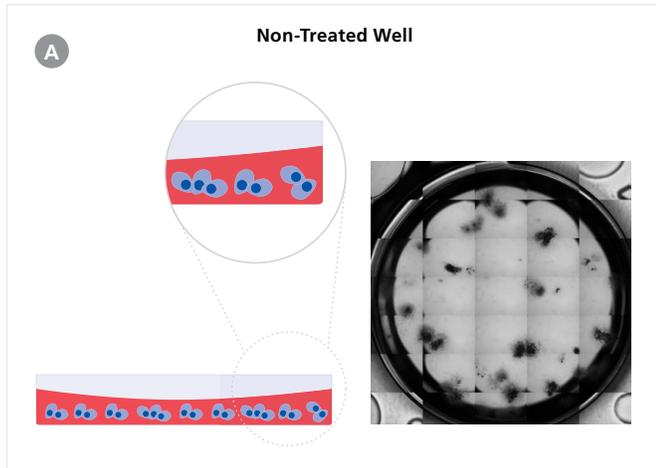


Figure 7. Mouse BM CFU Assays Performed in Standard Non-Treated and SmartDish™ 6-Well Culture Plates

Shown are representative STEMvision™ images of mouse BM CFU assays performed in 35 mm wells of either (A) non-treated or (B) SmartDish™ 6-well plates, after incubation for 12 days. The formation of a meniscus in standard cultureware causes more colonies to form around the periphery of the well where the culture medium is deeper. Optical distortion obscures 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 uniform distribution of colonies throughout each well.

Product Information

Product	Catalog #
STEMvision™ Instrument	22000/22000E

Software

Product	Catalog #
STEMvision™ Mouse Total CFU Analysis Package	22008
STEMvision™ Mouse Myeloid CFU Analysis Package	22009
STEMvision™ Mouse Erythroid CFU Analysis Package	22011

Buddles

Product	Catalog #
STEMvision™ Mouse Myeloid (22009) and Erythroid (22011) CFU Analysis Packages	22012
MethoCult GF™ M3534 (03534) and MethoCult™ SF M3436 (03436)	22013

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:

- 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

Storage Conditions:

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

For related products for mouse HSPC research, including specialized culture media, antibodies and cytokines, visit www.stemcell.com/HSPCworkflow or contact us at techsupport@stemcell.com.

Working with Human Tissues?

STEMvision™ Analysis Packages are also available for automated and standardized counting of CFU assays of human blood and bone marrow. Visit www.stemcell.com/STEMvision for more information.

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Automated and Standardized
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