TECHNICAL MANUAL

VERSION 1.4.0

STEMCELL Quality Control Kits

STEMCELL™ TECHNOLOGIES

For Research Use Only. Not For Therapeutic or Diagnostic Use.

In North America
Toll-Free T. 1.800.667.0322
Toll-Free F. 1.800.567.2899
T. 1.604.877.0713
F. 1.604.877.0704
E. info@stemcell.com
E. orders@stemcell.com

In Europe
Toll-Free T. 00.800.7836.2355
Toll-Free F. 00.800.7836.2300
T. +33 (0)4.76.04.75.30
F. +33 (0)4.76.18.99.63
E. info.eu@stemcell.com

In Australia
Toll-Free T. 1.800.060.350
F. +61 (03)9338.4320
E. info.aus@stemcell.com

In Singapore
T. 65.6776.7754
F. 65.6776.7114
E. info.sg@stemcell.com

STEMCELL Technologies
Version 1.4.0
November 2011
Catalog #28465

Table of Contents

1.0 Introduction 1
2.0 Thawing Cells, Plating and Colony Enumeration 2
2.1 Supplies and reagents included in the QC kit: 2
2.2 Additional reagents and equipment necessary to perform testing: 3
2.3 Storage of Products 3
2.4 Method 4
2.4.1 Thawing Cells 4
2.4.2 Performing Cell Counts 4
2.4.2.1. Manual Nucleated Cell Count 4
2.4.2.2. Viable Cell Count (Trypan Blue Exclusion Test) 5
2.4.3 Plating Colony Assays 6
2.4.4 Colony Enumeration 7
3.0 Option of $CD34^+$ Cell Enumeration 8
4.0 Worksheet 9

For Research Use Only. Not For Therapeutic or Diagnostic Use.

In North America
Toll-Free T. 1.800.667.0322
Toll-Free F. 1.800.567.2899
T. 1.604.877.0713
F. 1.604.877.0704
E. info@stemcell.com
E. orders@stemcell.com

In Europe
Toll-Free T. 00.800.7836.2355
Toll-Free F. 00.800.7836.2300
T. +33 (0)4.76.04.75.30
F. +33 (0)4.76.18.99.63
E. info.eu@stemcell.com

In Australia
Toll-Free T. 1.800.060.350
F. +61 (03)9338.4320
E. info.aus@stemcell.com

In Singapore
T. 65.6776.7754
F. 65.6776.7114
E. info.sg@stemcell.com

STEMCELL Technologies
Version 1.4.0
November 2011
Catalog #28465

In North America
Toll-Free T. 1.800.667.0322
Toll-Free F. 1.800.567.2899
T. 1.604.877.0713
F. 1.604.877.0704
E. info@stemcell.com
E. orders@stemcell.com

In Europe
Toll-Free T. 00.800.7836.2355
Toll-Free F. 00.800.7836.2300
T. +33 (0)4.76.04.75.30
F. +33 (0)4.76.18.99.63
E. info.eu@stemcell.com

In Australia
Toll-Free T. 1.800.060.350
F. +61 (03)9338.4320
E. info.aus@stemcell.com

In Singapore
T. 65.6776.7754
F. 65.6776.7114
E. info.sg@stemcell.com

STEMCELL Technologies
Version 1.4.0
November 2011
Catalog #28465

1.0 Introduction

Clonogenic assays are dependent on the ability of single hematopoietic progenitor cells to divide and differentiate, forming clusters of cells (colonies) in semi-solid media containing appropriate growth factors. Since their introduction more than 40 years ago, colony assays have been used extensively for research and clinical applications including identification of stimulatory and inhibitory growth factors, supportive diagnostic assays of myeloproliferative disorders and leukemias, and evaluation of the hematopoietic potential of bone marrow, leukapheresis and cord blood cell preparations for clinical transplantation.

The colony assay is the benchmark functional assay to assess the ability of various hematopoietic cell sources to divide and differentiate, especially following ex vivo manipulations including T-cell depletion, volume reduction, $CD34^+$ cell enrichment, cryopreservation, long-term storage, thawing and washing.

It is important to maintain a high degree of consistency in the progenitor assay set-up and readout (colony enumeration) within a given laboratory. The STEMCELL Quality Control Kits enable laboratories to monitor their consistency on a regular basis. Data generated in CFC-assays that are set up each month using cryopreserved samples of the same human cell preparation supplied with each STEMCELL Quality Control kit will provide a record of the laboratory's or individual technologist's reproducibility at setting up, culturing and enumerating hematopoietic colonies over the course of one year. Variations in the numbers of colonies scored in successive monthly assays can reveal inconsistencies in lab performance that may need to be addressed. In addition, large variations in colony numbers may highlight equipment malfunction (e.g. freezer or incubator) that may otherwise have gone undetected.

STEMCELL Quality Control kits are available with human cells from bone marrow (STEMCELL QC-BM) or cord blood (STEMCELL QC-CB) to allow assessment of the cell type most appropriate to a given laboratory's applications.

PRODUCT	CATALOG #	CELL TYPE PROVIDED IN KIT
STEMCELL QC-BM	00650	Human Bone Marrow
STEMCELL QC-CB	00651	Human Cord Blood

For Research Use Only. Not For Therapeutic or Diagnostic Use.

2.0 Thawing Cells, Plating and Colony Enumeration

2.1 Supplies and reagents included in the QC kit:

- Twelve frozen vials of pre-tested cells (5 x 10^6 cells per vial)

  The source of the cells will vary depending on the catalog number ordered

  Cryopreserved Bone Marrow, light density separated cells (Catalog #00650)

  Cryopreserved Cord Blood, light density separated cells (Catalog #00651)

- Twelve tubes of MethoCult™ GF H4034 Optimum (3 mL per tube)

  Product contains the following components:

  Methylcellulose in Iscove's Modified Dulbecco's Medium (IMDM)
  Fetal Bovine Serum (FBS)
  Bovine Serum Albumin (BSA)
  2-Mercaptoethanol
  rh Stem Cell Factor (SCF)
  rh Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF)
  rh Interleukin-3 (IL-3)
  rh Granulocyte Colony-Stimulating Factor (G-CSF)
  rh Erythropoietin (EPO)
  Supplements

- Fifteen sterile 16-gauge blunt-end needles
- Fifteen sterile 3 mL syringes
- Forty 35 mm tissue culture dishes
- Five 60 mm gridded scoring dishes
- Fifteen 100 mm dishes
- Twelve 100 mL bottles of IMDM + 2% FBS
- Trypan Blue (one 20 mL bottle)
- Colony Atlas
- Instruction Manual
- Letter, with lot-specific information about the optimal cell plating concentration

In North America
Toll-Free T. 1.800.667.0322
Toll-Free F. 1.800.567.2899
T. 1.604.877.0713
F. 1.604.877.0704
E. info@stemcell.com
E. orders@stemcell.com

In Europe
Toll-Free T. 00.800.7836.2355
Toll-Free F. 00.800.7836.2300
T. +33 (0)4.76.04.75.30
F. +33 (0)4.76.18.99.63
E. info.eu@stemcell.com

In Australia
Toll-Free T. 1.800.060.350
F. +61 (03)9338.4320
E. info.aus@stemcell.com

In Singapore
T. 65.6776.7754
F. 65.6776.7114
E. info.sg@stemcell.com

STEMCELL Technologies
Version 1.4.0
November 2011
Catalog #28465

2.2 Additional reagents and equipment necessary to perform testing:

- Class II laminar flow biohazard cabinet certified for Level II handling of human marrow and peripheral blood specimens
- Low speed centrifuge (e.g. Beckman TJ-6) equipped with biohazard containers for handling human cells
- 37°C incubator with humidity and gas control to maintain >95% humidity and an atmosphere of 5% CO_2 in air
- Vortex mixer
- Inverted microscope with flat field objectives and eyepieces to give total object magnification of approximately 20-30X, 40-63X and 100-125X.

  Note: Total object magnification = eyepiece x objective

  i.e. 25X = 2.5 x 10
- Hemacytometer (e.g. Neubauer)
- 3% acetic acid with methylene blue (Catalog #07060)
- Routine light microscope for hemocytometer cell counts
- 12 x 75 mm tube (e.g. Falcon #2058)
- 12 x 10 mm tube (e.g. Falcon #2057)
- 1 mL, 2 mL, and 10 mL pipettes
- Sterile distilled water
- Hand tally counter

2.3 Storage of Products

- Bone marrow and cord blood cells should be stored at -135°C or colder, or in liquid nitrogen. Cells can be stored for up to two years under these conditions.
- MethoCult™ GF H4034 and IMDM + 2% FBS should be stored at -20°C (-25°C to -15°C).
- All other materials and reagents should be stored at room temperature (15°C to 25°C).

For Research Use Only. Not For Therapeutic or Diagnostic Use.

In North America
Toll-Free T. 1.800.667.0322
Toll-Free F. 1.800.567.2899
T. 1.604.877.0713
F. 1.604.877.0704
E. info@stemcell.com
E. orders@stemcell.com

In Europe
Toll-Free T. 00.800.7836.2355
Toll-Free F. 00.800.7836.2300
T. +33 (0)4.76.04.75.30
F. +33 (0)4.76.18.99.63
E. info.eu@stemcell.com

In Australia
Toll-Free T. 1.800.060.350
F. +61 (03)9338.4320
E. info.aus@stemcell.com

In Singapore
T. 65.6776.7754
F. 65.6776.7114
E. info.sg@stemcell.com

2.4 Method

2.4.1 Thawing Cells

1. Thaw cells quickly in a 37°C water bath and wipe cryovial with 70% ethanol. Do not vortex cells at any time during the thawing procedure.
2. Gently transfer cells into a 15 mL conical test tube.
3. Slowly add 10 mL IMDM + 2% FBS dropwise while holding tube and gently swirling.
4. Gently invert tube to mix.
5. Spin down cells at 300 x *g* for ten minutes.
6. Discard supernatant and flick tube gently to resuspend the pellet.
7. Resuspend cells to a final volume of 1 - 2 mL.

2.4.2 Performing Cell Counts

2.4.2.1. Manual Nucleated Cell Count

1. Clean coverslip and hemacytometer thoroughly with alcohol. Dry coverslip and hemacytometer with lint-free tissue before using. Place the coverslip on the hemacytometer so that it is centered over both chambers
2. Dilute sample for a nucleated cell count according to your laboratory standards. Mix the diluted sample. Example: Place 20 µL of sample into 380 µL of 3% acetic acid with methylene blue (Catalog #07060) to achieve a 1:20 dilution.
3. Draw up an aliquot of diluted sample using a micropipettor or capillary tube.
4. Fill both chambers of the hemacytometer using a micropipettor or capillary tube. Do not overfill or underfill the chambers.
5. Starting with one chamber of the hemacytometer, count all the nucleated cells in at least two of the major corner 1 mm squares using a hand tally counter or other similar device. Count the same number of squares in the opposite chamber. Keep a total count of the cells and establish the average number of cells per square. If the cell count is less than 10 cells per square, a more concentrated suspension should be prepared (i.e. 20 µL of sample into 180 µL of 3% acetic acid with methylene blue to achieve a 1:10 dilution).
6. Determine the cell concentration as follows:

   Each of the nine major squares of the hemacytometer, with coverslip in place, represents a total volume of 0.1 mm^3 (or 10^{-4} cm^3 which is equivalent to 10^{-4} mL). The cell concentration and total number of cells can be determined using the following calculations:

   CELLS PER ML = the average count per square x the dilution factor x 10^4

   TOTAL CELLS = cells per mL x the original start volume

For Research Use Only. Not For Therapeutic or Diagnostic Use.

2.4.2.2. Viable Cell Count (Trypan Blue Exclusion Test)

1. Clean coverslip and hemacytometer thoroughly with alcohol. Dry coverslip and hemacytometer with lint-free tissues before using. Place the coverslip on the hemacytometer so that it is centered over both chambers.
2. Dispense 100 μL of cell suspension into a 12 x 75 mm tube.
3. Dispense 100 μL of trypan blue into the same tube.
4. Agitate gently and let tube stand undisturbed for two minutes. Do not let the mixture stand for longer than five minutes as viable cells may begin to take up the stain as well.
5. Draw up an aliquot of the diluted sample using a micropipettor or capillary tube.
6. Fill both chambers of the hemacytometer using a micropipettor or capillary tube. Do not overfill or underfill the chambers.
7. Using a multi-channel counter or two hand tally counters, score each viable, clear (non-blue) nucleated cell and each non-viable, blue nucleated cell (cells with damaged membranes) separately. Continue to score squares in the hemacytometer until you have a scored a minimum of 100 cells.
8. Calculate the percent viability using the following formulas:

   $$\% \text{ viability} = \frac{\text{total number of clear (non-blue) cells}}{\text{total number of clear (non-blue) cells + blue cells}} \times 100\ \%$$

9. For plating purposes, use the number of viable cells per mL to determine the cell concentration dilution. The number of viable cells per mL is calculated as follows:

   Cell count per mL x % viability

   Example: Viable cell per mL = cell count per mL x % viability
   = 3.8×10^6 cells per mL x 92%
   = 3.5×10^6 cells per mL

For Research Use Only. Not For Therapeutic or Diagnostic Use.

In North America
Toll-Free T. 1.800.667.0322
Toll-Free F. 1.800.567.2899
T. 1.604.877.0713
F. 1.604.877.0704
E. info@stemcell.com
E. orders@stemcell.com

In Europe
Toll-Free T. 00.800.7836.2355
Toll-Free F. 00.800.7836.2300
T. +33 (0)4.76.04.75.30
F. +33 (0)4.76.18.99.63
E. info.eu@stemcell.com

In Australia
Toll-Free T. 1.800.060.350
F. +61 (03)9338.4320
E. info.aus@stemcell.com

In Singapore
T. 65.6776.7754
F. 65.6776.7114
E. info.sg@stemcell.com

STEMCELL Technologies
Version 1.4.0
November 2011
Catalog #28465

2.4.3 Plating Colony Assays

1. Thaw a tube of MethoCult™ GF H4034 under refrigeration (2 - 8°C) overnight or at room temperature (15 - 25°C).
2. Refer to the letter (included with the kit) containing information for the cell concentration required to prepare the working cell suspension used in setup of the CFC-assay. Note that the cell plating concentration may be different between different lots of the kit. Always use the cell concentration provided in the letter included with the same kit you are using. After diluting and dispensing the final cell concentration per 35 mm dish will be 1/10 of the cell concentration in the working cell suspension.
3. Calculate the volume of cell stock and IMDM with 2% FBS required to make 1 mL of the working cell suspension:

   Volume of stock cell suspension needed (mL) =

   $$\frac{\text{Cell concentration of the working cell suspension (provided in the letter)}}{\text{Viable Cell Concentration (calculated in step 9 of the previous paragraph)}}$$

   Volume of IMDM + 2% FBS (mL) = (1 mL) – (Volume of stock cell suspension (mL))
4. Gently mix the **Volume of stock cell suspension needed + Volume of IMDM with 2% FBS** (as calculated above) to prepare the working cell suspension for CFC-assay set up.
5. Add 0.3 mL of the working cell suspension to the 3 mL tube of MethoCult™ GF H4034.
6. Vortex the tube vigorously for at least three seconds. After vortexing, let the tube stand for at least five minutes to allow all bubbles to rise to the surface.
7. Prepare 35 mm petri dishes by placing them in pairs inside a 100 mm petri dish. Be sure to add a third 35 mm dish (without its lid) for a water dish. The purpose of the water dish is to ensure that maximum humidity is maintained during incubation. The 35 mm dishes used for the assay cultures have been pre-tested for optimal colony growth and do not support growth of anchorage-dependent cells.

   The package of ten 35 mm dishes should be resealed for future assays.
8. To plate the MethoCult™ GF H4034 and cell mixture into the sterile petri dishes, attach a 16-guage blunt-end needle to a 3 mL syringe. Draw up the methylcellulose and cell mixture to the 1.0 mL mark and slowly dispense this initial volume back into the tube in order to remove the large air bubble that is present in the syringe and needle. Draw up the mixture again to the 2.6 mL mark. Dispense 1.1 mL of methylcellulose into a labelled 35 mm petri dish (plunger now at 1.5 mL mark). Dispense another 1.1 mL into the second labelled petri dish (plunger now at 0.4 mL mark).
9. Rotate and tilt each dish to spread the viscous methylcellulose mixture evenly across the surface of each dish.
10. Add 3 mL of sterile water to the water dish. Place the cultures on a level tray in a 37°C humidified incubator with 5% CO_2 in air. It is important that the correct temperature, CO_2 and humidity (>95%) are maintained in the incubator during entire culture period.
11. Incubate for 14 days at 37°C, 5% CO_2 in air and >95% humidity.

For Research Use Only. Not For Therapeutic or Diagnostic Use.

In North America
Toll-Free T. 1.800.667.0322
Toll-Free F. 1.800.567.2899
T. 1.604.877.0713
F. 1.604.877.0704
E. info@stemcell.com
E. orders@stemcell.com

In Europe
Toll-Free T. 00.800.7836.2355
Toll-Free F. 00.800.7836.2300
T. +33 (0)4.76.04.75.30
F. +33 (0)4.76.18.99.63
E. info.eu@stemcell.com

In Australia
Toll-Free T. 1.800.060.350
F. +61 (03)9338.4320
E. info.aus@stemcell.com

In Singapore
T. 65.6776.7754
F. 65.6776.7114
E. info.sg@stemcell.com

STEMCELL Technologies
Version 1.4.0
November 2011
Catalog #28465

2.4.4 Colony Enumeration

1. Prepare a 60 mm gridded scoring dish by drawing two perpendicular lines across the center of the dish using a permanent fine felt marker on the bottom of the dish (refer to "Atlas of Human Hematopoietic Colonies", page 4). This scoring dish can be used again to score other culture dishes.
2. Remove cultures from the incubator and place them (with the lid still on), one at a time, inside the 60 mm gridded tissue culture dish to score the colonies in situ using an inverted microscope. Scoring is usually easier if colonies are counted in vertical rows by moving the microscope stage up and down (rather than across) the dish.
3. Counts at day 14 - 16 should include the smaller erythroid colonies, derived from the most mature types of erythroid colony-forming cells (i.e. from CFU-E); the larger erythroid colonies (from primitive BFU-E); all granulopoietic colonies (from CFU-GM); and colonies containing multiple lineages of cells (from CFU-GEMM). For detailed assistance in the recognition of various colony types, refer to the "Atlas of Human Hematopoietic Colonies" provided.

CFU-E	Colony-Forming Unit-Erythroid of 1-2 small clusters containing 8 - 200 erythroblasts
BFU-E	Burst-Forming Unit-Erythroid containing greater than 200 erythroblasts (may contain greater than 2 clusters)
CFU-GM	Colony-Forming Unit-Granulocyte, Macrophage containing 40 or more cells of the granulocyte and/or macrophage lineage
CFU-GEMM	Colony-Forming Unit-Granulocyte, Erythroid, Macrophage, Megakaryocyte containing erythroid cells and 20 or more granulocyte, macrophage, erythroid and megakaryocyte cells

   Note: The use of a blue filter enhances the red color of the erythroid colonies and may help in the identification of both CFU-E and BFU-E.
4. Scan the entire dish at a magnification of 20 - 30X to ensure that the plating efficiency is representative of the entire dish. Scoring or counting the number and types of colonies is best done using an inverted microscope equipped with high quality flatfield objectives and eyepieces to give total object magnification of approximately 20 - 30X, 40 - 63X and 100 - 125X.

   Total object magnification = eyepiece multiplied by the objective.

   Example: 62.5X = 12.5 x 5
5. Score the CFU-E using the total object magnification 40 - 63X. Once completed, score the remaining BFU-E, CFU-GEMM and CFU-GM colonies using the 20 - 30X total object magnification. Use a higher magnification to confirm colony type if uncertain.
6. Record the number of colonies in each of the culture dishes on the worksheet provided at the back of this manual in Section 4.0. Calculate the average number of each type of progenitor detected in the colony assay by dividing the sum of the number of colonies in the two dishes by two. Keep a copy of this Worksheet in your files for future use.

3.0 Option of $CD34^+$ Cell Enumeration

Materials and recommendations for $CD34^+$ cell enumeration have not been supplied with this kit. However, the cell suspension may be tested to assess the consistency of $CD34^+$ cell enumeration by flow cytometry using the standard operating procedures and antibodies employed in your laboratory.

The $CD34^+$ cell frequency determined following incubation of conjugated antibodies and flow cytometry analysis should be recorded for each test. Plots generated from the monthly analysis of $CD34^+$ cell frequency will demonstrate the reproducibility of your protocol to enumerate the $CD34^+$ cell content of a given sample.

For Research Use Only. Not For Therapeutic or Diagnostic Use.

In North America
Toll-Free T. 1.800.667.0322
Toll-Free F. 1.800.567.2899
T. 1.604.877.0713
F. 1.604.877.0704
E. info@stemcell.com
E. orders@stemcell.com

In Europe
Toll-Free T. 00.800.7836.2355
Toll-Free F. 00.800.7836.2300
T. +33 (0)4.76.04.75.30
F. +33 (0)4.76.18.99.63
E. info.eu@stemcell.com

In Australia
Toll-Free T. 1.800.060.350
F. +61 (03)9338.4320
E. info.aus@stemcell.com

In Singapore
T. 65.6776.7754
F. 65.6776.7114
E. info.sg@stemcell.com

STEMCELL Technologies
Version 1.4.0
November 2011
Catalog #28465

4.0 Worksheet

STEMCELL QC LOT#		CFU-E	BFU-E	CFU-GM	CFU-GEMM	Total CFC
Test 1	Plate 1					
	Plate 2					
	Average					
Test 2	Plate 1					
	Plate 2					
	Average					
Test 3	Plate 1					
	Plate 2					
	Average					
Test 4	Plate 1					
	Plate 2					
	Average					
Test 5	Plate 1					
	Plate 2					
	Average					
Test 6	Plate 1					
	Plate 2					
	Average					
Test 7	Plate 1					
	Plate 2					
	Average					
Test 8	Plate 1					
	Plate 2					
	Average					
Test 9	Plate 1					
	Plate 2					
	Average					
Test 10	Plate 1					
	Plate 2					
	Average					
Test 11	Plate 1					
	Plate 2					
	Average					
Test 12	Plate 1					
	Plate 2					
	Average					

For Research Use Only. Not For Therapeutic or Diagnostic Use.

In North America
Toll-Free T. 1.800.667.0322
Toll-Free F. 1.800.567.2899
T. 1.604.877.0713
F. 1.604.877.0704
E. info@stemcell.com
E. orders@stemcell.com

In Europe
Toll-Free T. 00.800.7836.2355
Toll-Free F. 00.800.7836.2300
T. +33 (0)4.76.04.75.30
F. +33 (0)4.76.18.99.63
E. info.eu@stemcell.com

In Australia
Toll-Free T. 1.800.060.350
F. +61 (03)9338.4320
E. info.aus@stemcell.com

In Singapore
T. 65.6776.7754
F. 65.6776.7114
E. info.sg@stemcell.com

STEMCELL Technologies
Version 1.4.0
November 2011
Catalog #28465

TOLL-FREE PHONE 1 800 667 0322 • PHONE 1 604 877 0713

TOLL-FREE FAX 1 800 567 2899 • FAX 1 604 877 0704

ORDERS@STEMCELL.COM • INFO@STEMCELL.COM

FOR FULL CONTACT DETAILS WORLDWIDE VISIT OUR WEBSITE

FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE. MANUAL CATALOG #28465 VERSION 1.4.0

Copyright © 2011 by STEMCELL Technologies Inc. All rights reserved, including graphics and images. STEMCELL Technologies & Design, STEMCELL shield, Scientists Helping Scientists and MethoCult are trademarks of STEMCELL Technologies Inc. All other trademarks and registered trademarks are the property of their respective holders.