Proficiency Testing: Instructions for Frozen Samples

Ordering Information

Catalog #100-0926/100-0928 #100-0927/100-0929 1 Kit (Frozen Human Bone Marrow) Additional Kits

OR

Catalog #100-0950/100-0952 #100-0951/100-0953 1 Kit (Frozen Cord Blood) Additional Kits

Component Information

COMPONENT NAME	COMPONENT #	SIZE	CONDITION UPON RECEIPT	STORAGE
Human Bone Marrow Mononuclear Cells, Frozen OR Human Cord Blood Mononuclear Cells, Frozen	00309 OR 00312	1 mL	Frozen, on dry ice OR liquid nitrogen	Store at -135°C or colder.
MethoCult™ GF	04050	5 mL	Frozen, on dry ice	Store at -20°C.
Iscove's MDM with 2% FBS	07700	100 mL	Frozen, on dry ice	Store at -20°C.
Dry Goods Kit	00620	1 kit	Room temperature	Store at room temperature (15 - 25°C).
Letter, Session-Specific Information (Frozen Human Bone Marrow) OR Letter, Session-Specific Information (Frozen Human Cord Blood)	N/A	1 letter	Room temperature	Not applicable.

Procedure

Verify all materials have arrived according to the Condition Upon Receipt outlined in the above table. Any deviation from these receipt conditions should be immediately reported to Product and Scientific Support (1.800.667.0322 or techsupport@stemcell.com). Set up your Proficiency Testing sample immediately upon receipt of the kit to ensure that your data is submitted on time for inclusion in the cohort analysis. For detailed instructions on cell processing and colony assay setup, refer to the Technical Manual: Human Colony-Forming Unit (CFU) Assays Using MethoCult™, available at www.stemcell.com.

Test 1 – Cell Preparation

DEFINITIONS

Cell Stock: The mononuclear cell sample washed with Iscove's MDM with 2% FBS.

Viable Cell Concentration: The **Nucleated Cell Concentration** of the **Cell Stock** times **% Viability**.

10X Plating Density: The cell concentration used to set up the colony-forming unit (CFU) assay with a predetermined number of viable cells. Refer to the session-specific letter included in the Proficiency Testing Kit. The **Cell Stock** is diluted in Iscove's MDM with 2% FBS to equal ten times (10X) the **Final Plating Density**.

Final Plating Density: The number of viable cells per volume of semi-solid culture medium per well.

CELL COUNTING

NOTE: Thaw MethoCult™ and Iscove's MDM with 2% FBS at room temperature (15 - 25°C) or at 2 - 8°C overnight prior to setting up the assay. Bring to room temperature before use.

Aim to complete this entire procedure, including cell preparation and inoculation, within 1 hour. The cell counting procedures outlined in steps 8 - 9 are suggestions. Use procedures that have been validated in your institution.

1. Thaw cells quickly (approximately 2 minutes) in a 37°C water bath. When the cells are almost completely thawed, wipe cryovial with 70% ethanol or 70% isopropanol.
2. Gently transfer cells to an empty 15 mL polystyrene or polyethylene terephthalate (PET) tube.
3. Slowly (dropwise) add 10 mL Iscove's MDM with 2% FBS while gently swirling tube (approximately 1 minute). Gently invert the tube 3 - 4 times to mix. Do not vortex.
4. Centrifuge at 300 x *g* for 10 minutes at room temperature (15 - 25°C) with the brake on. Using a serological pipette, carefully remove the supernatant, taking care not to dislodge the cell pellet. Do not pour off supernatant.
5. Resuspend the cells in the residual volume of medium by gently flicking the tube.

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6. Add 2 mL of Iscove's MDM with 2% FBS and measure the total volume in mL. Record the **Volume of Cells** in Test 1, Row A.
7. Using a serological pipette, gently mix the **Cell Stock**.
8. Perform a nucleated cell count on the **Cell Stock**. A suggested procedure using 3% acetic acid is outlined in section 8.1 of the Technical Manual (Document #28404). Record the result in Test 1, Row B as the **Nucleated Cell Concentration** in 10^6 cells/mL.

   NOTE: Do not multiply the cell concentration value by the total volume for this field.
9. Perform a viable cell count on the **Cell Stock**. A suggested procedure using trypan blue dye exclusion is outlined in section 8.2 of the Technical Manual (Document #28404). Record the viable cell count (unstained cells) and non-viable cell count (stained cells) for the **Cell Stock** and calculate **% Viability** using the formula below. Record the result in Test 1, Row C.

$$\% \text{ Viability} = \frac{\text{viable cell count}}{\text{(viable + non-viable cell count)}} \times 100\%$$

DILUTION OF CELL STOCK

The following steps outline how to dilute the **Cell Stock** to prepare the **10X Plating Density**, which is then diluted ten-fold in MethoCult™ to generate the **Final Plating Density**.

10. Use the following formula to calculate the **Viable Cell Concentration** of the **Cell Stock**:

$$\text{Viable Cell Concentration} = \text{Nucleated Cell Concentration (Test 1, Row B)} \times \text{\% Viability (Test 1, Row C)}$$

11. Use the following formulas to calculate the **Volume of Cell Stock** and the **Volume of Iscove's MDM + 2% FBS** required to make 1 mL of the **10X Plating Density**:

$$\text{Volume of Cell Stock (mL)} = \frac{\text{10X Plating Density (refer to Document \#29115 or 29116 [cells/mL])} \times 1 \text{ mL}}{\text{Viable Cell Concentration (refer to step 10 [cells/mL])}}$$

$$\text{Volume of Iscove's MDM + 2\% FBS (mL)} = (1 \text{ mL}) - (\text{Volume of Cell Stock [mL]})$$

12. Prepare the **10X Plating Density** by gently mixing the **Cell Stock** and Iscove's MDM + 2% FBS (volumes calculated in step 11).

CELL INOCULATION

13. Prepare the **Final Plating Density** by adding 0.5 mL of the **10X Plating Density** to the 5 mL tube of MethoCult™ and vortex vigorously for at least 4 seconds. Let stand at least 5 minutes.
14. Using the syringe and blunt-end needle provided, plate 1.1 mL of **Final Plating Density** into each of 4 x 35 mm culture dishes. Refer to the Technical Manual (Document #28404) for details on dispensing MethoCult™ using a syringe.

    NOTE: The statistical analysis requires data from all 4 replicates.
15. Cover the dishes with lids and gently swirl to completely coat the bottoms of each.
16. Place 2 x 35 mm dishes containing **Final Plating Density** in each of the 100 mm dishes. Add a third 35 mm dish containing sterile water (with the lid removed) to each 100 mm dish to ensure adequate humidity. Cover both of the 100 mm dishes. Extra 35 mm dishes are provided.
17. Incubate at 37°C, 5% CO_2, ≥ 95% humidity for 14 days.

Test 2 – Colony Enumeration

Count colonies on day 14 and record results on page 3 (Test 2 - Colony Enumeration Data). Enter N/A for unreported values. Empty cells will also be interpreted as unreported values. Enter 0 to indicate the absence of colonies.

For detailed assistance in colony identification, refer to either the Atlas of Human Hematopoietic Colonies (Catalog #28700 [for BM]) or the Atlas of Hematopoietic Colonies From Cord Blood (Catalog #29940 [for CB]), **available in PDF under "Educational Materials" on the Proficiency Testing webpage.**

NOTE: The statistical analysis requires data from all 4 replicates. Unreported values will prevent that parameter from being added to the statistical analysis.

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Data Submission Worksheet

You can submit data in one of three ways:

- **Online** with the Proficiency Testing Data Submission Forms available at www.proficiencytesting.com. Ensure you select the online data submission form that corresponds with the appropriate program.
- **Email** this completed worksheet to proficiency@stemcell.com.

Name:_____ Email:_____

Institution:_____ Participant ID:_____

Test 1 – Cell Preparation Data

CELL COUNTING AND VIABILITY RESULTS		
A	Volume of Cells (mL)	
B*	Nucleated Cell Concentration (10^6 cells/mL)	
C	% Viability	

*Submit cell concentration per mL. Do not multiply the cell concentration value by the total volume.

CELL COUNTING METHOD								
Method (circle one response)	Automatic				Manual			
Dye Used	Trypan Blue	Acetic Acid	7-AAD	AO	PI	AO/PI	Other	None
Instrument Used (automatic only)								

VIABILITY ASSESSMENT METHOD							
Method (circle one response)	Automatic			Manual			
Dye Used	Trypan Blue	7-AAD	AO	PI	AO/PI	Other	None
Instrument Used (automatic only)							

Is this method routinely used in your laboratory?

Yes	No

CB only: For automated methods, do you adjust for nucleated red blood cells?

Yes	No

Resources

The video "CFU Assay Instructions for Global Proficiency Testing Programs" is available at **www.stemcell.com/proficiencyvideo**.

A variety of other resources are available to assist you at **www.stemcell.com/technical-resources.html**.

Test 2 – Colony Enumeration Data

(A) If you distinguish all colony types, complete the first table below and leave tables (B) and (C) blank.

COLONY TYPE	DISH			
	1	2	3	4
CFU-E (BM only)				
BFU-E				
CFU-GM				
CFU-GEMM				

(B) If you pool the values for CFU-E and BFU-E colonies (rather than distinguishing these colony types), leave the fields in table (A) for CFU-E and BFU-E blank (--) and enter your values in table (B) below.

Total Erythroid (BM only)				

(C) If you only report total colony counts, leave the above tables (A) and (B) blank and complete the table below.

Total Colonies				

Test 3 – Colony Identification Data

Identify the colonies in photographs A - H, found within the data submission form at **www.proficiencytesting.com**.

PHOTO	COLONY	PHOTO	COLONY
A		E	
B		F	
C		G	
D		H	

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