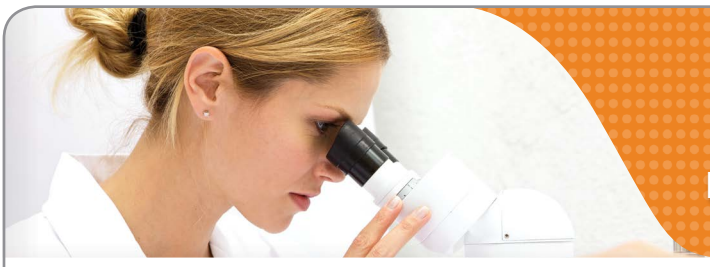

Proficiency Testing

Instructions for Frozen Cord Blood Program

Receipt of Proficiency Testing Kit (Catalog #00608/00609)

CATALOG #	DESCRIPTION	CONDITION UPON RECEIPT	STORAGE CONDITIONS
00312	Frozen Human Cord Blood Mononuclear Cells	Frozen, on dry ice	-135°C or colder (or in Liquid N_2)
04050	MethoCult™ GF for PT	Frozen, on dry ice	-20°C (-25°C to -15°C)
07700	IMDM + 2% FBS	Frozen, on dry ice	-20°C (-25°C to -15°C)
00620	Dry Goods Kit	Room temperature	Room temperature (15 - 25°C)
29116	Letter, Session-Specific Information CB	Room temperature	Required for assay setup

Procedure

Verify all materials have arrived according to the "Condition Upon Receipt" outlined in the above table. Any deviation from these shipping conditions should be immediately reported to our Product and Scientific Support department (1-800-667-0322 or techsupport@stemcell.com). Please set up your Proficiency Testing sample as soon as possible after receipt of the kit to ensure that your data is submitted on time to be included in the group analysis. If you cannot set up the assay on the day of receipt, move all materials to the appropriate "Storage Conditions" outlined in the above table. For detailed instructions on cell processing and colony assay set up, refer to the Technical Manual for Human CFU Assays Using MethoCult™ (Document #28404), available at www.stemcell.com.

Definitions

Cell Stock: the mononuclear cell sample washed with IMDM + 2% FBS.
Viable Cell Concentration: the nucleated cell concentration of the Cell Stock times the percent (%) Viability.
10X Plating Density: the cell concentration used to set up the CFU assay with a predetermined number of viable cells. Refer to the session-specific information letter (Catalog #29116) included in the Proficiency Testing kit. The Cell Stock is diluted in IMDM + 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.

Test 1 – Cell Preparation

Cell Counting

Aim to complete this entire procedure including cell preparation and inoculation within 1 hour. Ensure the MethoCult™ GF and IMDM + 2% FBS have been thawed overnight prior to setting up the assay. The cell counting procedures outlined in steps 8 - 10 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 tube.
3. Slowly (dropwise) add 10 mL IMDM + 2% FBS while gently swirling tube (approximately 1 minute). Gently invert the tube several times to mix. Do not vortex.
4. Centrifuge at 300 x *g* for 10 minutes at room temperature (15 - 25°C). Carefully remove the supernatant, taking care not to dislodge the cell pellet. Do not pour off.
5. Resuspend the cells in the remaining volume of medium by gently flicking the tube.
6. Add 2 mL of IMDM + 2% FBS and measure total volume. Record the volume *(Test 1, Row A)*.
7. Gently mix the cell stock.
8. Perform a nucleated cell count on the cell stock. A suggested procedure using acetic acid is outlined in section 8.1 of the Technical Manual (Document #28404).
9. Record the results from the nucleated cell count as the Nucleated Cell Concentration using the format 10^6 cells per mL *(Test 1, Row B). (Please do not multiply the cell concentration value by the total volume for this field).*
10. 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 the percent (%) Viability using the following formula *(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 the semi-solid culture medium to generate the Final Plating Density.

11. Use this formula to calculate the Viable Cell Concentration of the Cell Stock:

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

12. Use these formulas to calculate the volume of cell stock and volume of IMDM + 2% FBS required to make 1 mL of the 10X Plating Density:

$$\text{Volume of Cell Stock (mL)} = \frac{\text{10X Plating Density (refer to Catalog \#29116 (cells per mL))} \times 1\ \text{mL}}{\text{Viable Cell Concentration (refer to step 11 (cells per mL))}}$$

$$\text{Volume of IMDM + 2\% FBS (mL)} = (1\ \text{mL}) - (\text{Volume of Cell Stock (mL)})$$

13. Prepare the 10X Plating Density by gently mixing the cell stock and IMDM + 2% FBS.

Cell Inoculation

14. Prepare the Final Plating Density by adding 0.5 mL of the 10X Plating Density to the 5 mL tube of MethoCult™ GF and **vortex vigorously for at least 4 seconds**. Let stand at least 5 minutes.
15. Using the syringe and blunt-end needle provided, plate 1.1 mL of Final Plating Density into each of the four 35 mm culture dishes. Use a new syringe and blunt-end needle between duplicates to prevent contamination. Refer to the Technical Manual (Document #28404) for details on dispensing MethoCult™ GF using a syringe. **Note that the statistical analysis requires data from all four replicates.**
16. Cover the dishes with lids and gently swirl to completely coat the bottoms of each.
17. Place two 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.
18. Incubate at 37°C, 5% CO_2, ≥ 95% humidity for 14 days.

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FOR GLOBAL CONTACT DETAILS WORLDWIDE VISIT OUR WEBSITE

Name: _____ Email: _____

Institution: _____ Participant ID: _____

Data Submission

You can submit data in one of three ways:

- **Online** with the Proficiency Testing Data Submission Forms available on our website at www.proficiencytesting.com. Please ensure you select the online data submission form that corresponds with the **frozen cord blood** program as indicated at the top of the form.
- **E-mail** this completed worksheet to proficiency@stemcell.com
- **Fax** this completed worksheet to **1.604.877.0704** or **1.800.567.2899 (North America)** (Attention Education Department)

Test 1 - Cell Preparation

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

*In Row B, please submit cell concentration per mL. Do not multiply the cell concentration value by the total volume for this field.

CELL COUNTING METHOD						
Method (circle one response)	Automatic			Manual		
Dye Used	Trypan Blue	Acetic Acid	7AAD	AO	PI	Other
Instrument Used (not applicable for manual methods)						

VIABILITY ASSESSMENT METHOD					
Method (circle one response)	Automatic	Manual			
Dye Used	Trypan Blue	7AAD	AO	PI	Other
Instrument Used (not applicable for manual methods)					

Are these methods routinely used in your laboratory?

Yes	No

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

Yes	No

Test 2 – Colony Enumeration

Instructions

Count colonies on day 14 and record results below. 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 the Atlas of Human Hematopoietic Colonies (Document #29940). This is provided to each individual the first time they participate in the Proficiency Testing Program.

Note

Please note that the statistical analysis requires data from all four replicates. Unreported values will prevent that parameter from being added to the statistical analysis.

(A) If you distinguish all colony types please complete the first table below and leave section (B) blank.

COLONY	DISH			
	1	2	3	4
BFU-E				
CFU-GM				
CFU-GEMM				

(B) If you are only reporting total colony counts, please leave the above section (A) blank and complete the table below.

TOTAL COLONIES				

Test 3 - Colony Identification

Identify the colonies in photographs A – H on our website www.proficiencytesting.com.

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

A variety of resources are available to assist you at www.stemcell.com/en/Technical-Resources.