Proficiency Testing: Instructions for Fresh Cord Blood

Ordering Information

Catalog #00606 1 Kit (Fresh Cord Blood)
#00607 Additional Kits

Component Information

COMPONENT NAME	COMPONENT #	SIZE	CONDITION UPON RECEIPT	STORAGE
Fresh Human Cord Blood Cells for PT	00313	1 mL	Room temperature	Store at room temperature (15 - 25°C).
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).

Procedure

Verify all materials have arrived according to the Condition Upon Receipt section 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™ (Document #28404), available at **www.stemcell.com**.

Test 1 – Cell Preparation

DEFINITIONS

Cell Stock: The cell sample received in the Proficiency Testing (PT) kit.

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 dilution instructions provided by email from STEMCELL Technologies on the day the kit was received. 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.

DILUTION OF CELL STOCK

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. Dilute the **Cell Stock** to prepare the **10X Plating Density** as directed by the emailed instructions from STEMCELL Technologies. Keep the leftover undiluted cells (cell stock) for cell counting (see steps 8 - 9) and CD34+ enumeration.
2. Using a serological pipette, mix the cell suspension by gently pipetting up and down 3 - 4 times.

CELL INOCULATION

3. 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 (see Figure 1).
4. 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.
5. Cover the dishes with lids and gently swirl to completely coat the bottoms of each.

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6. 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 (see Figure 2). Cover both of the 100 mm dishes. Extra 35 mm dishes are provided.

   NOTE: Do not cover the water dish.

7. Incubate at 37°C, 5% CO_2, ≥ 95% humidity for 14 days.

0 minutes 5 minutes

Figure 1. Images of Bubbles Dissipating After Vortexing

Figure 2. 35 mm Dish Hydration

CELL COUNTING

8. Perform a nucleated cell count on the leftover **Cell Stock**. Before proceeding, mix the Cell Stock solution well. 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 A.
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). Determine 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 B.

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

Test 1 – Cell Preparation Data

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

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

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

Yes	No

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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.
- **Fax** this completed worksheet to 1.604.877.0704 or 1.800.567.2899 (North America) (Attention Education Department).

Name:_____

Email:_____

Institution:_____

Participant ID:_____

CD34+ Enumeration Data

CD34+ enumeration is optional for non-NMDP members.

CD34+ ENUMERATION METHOD AND RESULTS		
Instrument Used (to perform flow cytometric analysis)		
Platform Used (circle one response)	Double Platform	Single Platform
Methodology Used (circle one response)	ISHAGE Protocol	Modified ISHAGE Protocol
	Other (describe)	
Reagents (list antibodies used for phenotyping)	CD34+	
	CD45+	
	Other (e.g. name of commercially available kits)	
% CD34+ (gated on viable cells)		
% CD45+CD34+ (gates on viable cells)		

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

Count colonies on day 14 and record results below. Enter N/A for unreported values. Blank 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 Hematopoietic Colonies From Cord Blood (Catalog #29940), provided to each institute the first time they participate in the Proficiency Testing Program.

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

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

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

(B) If you only report total colony counts, leave the above table 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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