## Identification of Colonies Derived from

# Human Hematopoietic Progenitor Cells

Progenitor Cells	Description of Colonies Derived from Progenitor Cells	Cell Source Differences	Colonies
CFU-GEMM (Colony-forming unit – granulocyte, erythroid, macrophage, megakaryocyte)	<ul> <li>A colony containing both erythroid (hemoglobinized) cells and 20 or more non-erythroid (not hemoglobinized) cells, i.e. granulocytes, macrophages, and/or megakaryocytes</li> <li>Typically has erythroid cells in the center and non-erythroid cells around the periphery, but non-erythroid cells can be concentrated on one side of the erythroid cells</li> <li>Colonies derived from CFU-GEMM are usually larger than colonies derived from CFU-GM or BFU-E</li> <li>Relatively infrequent in most cell samples (typically &lt; 10% of total colonies)</li> </ul>	The frequency tends to be higher in cord blood and mobilized peripheral blood than in bone marrow, but variation between samples can be large	Image: A constrained of the constra
CFU-GM (Colony-forming unit – granulocyte, macrophage)	<ul> <li>A colony containing more than 20 granulocytes and/or macrophages</li> <li>Does not appear red or brown (i.e. cells are not hemoglobinized)</li> <li>Individual cells can usually be distinguished, particularly at the edge of the colony</li> <li>Large colonies may have one or more dense dark cores</li> <li>Does not require erythropoietin (EPO) for growth and differentiation</li> </ul>	Are generally larger in cord blood samples than in bone marrow or (mobilized) peripheral blood	4X Objective         4X Objective
BFU-E (Burst-forming unit – erythroid)	<ul> <li>A colony containing more than 200 erythroblasts in single or multiple clusters</li> <li>Appears red or brown, as the cells are hemoglobinized</li> <li>Difficult to distinguish individual cells within each cluster</li> <li>Develops in medium containing EPO and other cytokines, particularly interleukin-3 (IL-3) and stem cell factor (SCF)</li> </ul>	Are generally larger and have more clusters in cord blood than in bone marrow or (mobilized) peripheral blood	Image: Ax ObjectiveImage: Ax ObjectiveImage: Ax ObjectiveImage: Ax ObjectiveImage: Ax ObjectiveImage: Ax Objective
<section-header><text></text></section-header>	<ul> <li>A colony containing 8 – 200 erythroblasts in one to two small clusters</li> <li>Appears red or brown, as the cells are hemoglobinized</li> <li>Difficult to distinguish individual cells within the colony</li> <li>Develops in medium that contains EPO</li> </ul>	More frequent in bone marrow than in umbilical cord blood and (mobilized) peripheral blood	10X Objective 10X Objective

#### Human MethoCult<sup>™</sup> Formulations

PRODUCT NAME	CATALOG #	FORMAT	PROGENITOR CELLS DETECTED	SCF, GM- CSF, IL-3	G-CSF	IL-6	EPO
	04034	100 mL	CFU-E, BFU-E, CFU-GM, CFU-GEMM	٠			
MethoCult <sup>IIII</sup> H4034 Optimum	04044	24 x 3 mL			•		•
MethoCult™ H4035 Optimum without EPO	04035	100 mL	CFU-GM	•	٠		
MethoCult™ H4434 Classic	04434	100 mL	CFU-E, BFU-E, CFU-GM, CFU-GEMM			_	
	04444	24 x 3 mL		•			•
MethoCult™ H4534 Classic without EPO	04534	100 mL	CFU-GM	٠			
	04544	24 x 3 mL					
	04435	100 mL	CFU-E, BFU-E, CFU-GM, CFU-GEMM	٠	٠	٠	•
MethoCult <sup>1</sup> H4435 Enriched	04445	24 x 3 mL					
MethoCult™ H4535 Enriched	04535	100 mL	- CFU-GM	•			
without EPO	04545	24 x 3 mL			•	•	

### The Hematopoietic Hierarchy

Representation of production of mature cells from stem and progenitor cells.

Other MethoCult™ formulations, including serum-free formulations and formulations that allow the addition of cytokines, are available. Visit our website at **www.stemcell.com** for more information.

#### Other Equipment Required to Perform Colony Assays

PRODUCT DESCRIPTION	CATALOG #	QUANTITY	APPLICATIONS				
lscove's Modified Dulbecco's Medium (IMDM) with 2% Fetal Bovine Serum	07700	100 mL	Washing hematopoietic cells				
Dunt End Needles	28110	100	<ul> <li>Aliquoting MethoCult<sup>™</sup> and plating cultures</li> </ul>				
Blunt-end Needles	28120	2000	Recommended for prevention of needle-stick injuries				
	28230	30	Aliquoting MethoCult <sup>™</sup> and plating cultures				
3 cc Syringes	28240	100	<ul> <li>Syringes and blunt-end needles are recommended when dispensing MethoCult™</li> <li>The semi-solid medium will stick to the inside of a standard pipette, resulting in a less accurate volume</li> </ul>				
35 mm Dishes for Culture in	27100	10	- Optimal colony growth without supporting adherent colls				
MethoCult™	27150	500	• Optimal colorly growth without supporting adherent cells				
CO rome Criddod Cooring Dish	100-0085	20	• A 35 mm culture dish fits inside the 60 mm gridded scoring dish, which is a standard size for most microscope stages				
ou min Gridded scoring Disnes	38068	500	• Allows for reproducible and accurate scoring of colonies by ensuring areas of the dish are not counted more than once or not missed				

A high-quality inverted microscope with 2X, 4X, and 10X planar objectives, a stage holder for a 60 mm gridded dish, and a blue filter are required. A differential counter allows colonies of different lineages to be counted at the same time.



\*The colony-forming unit – megakaryocyte (CFU-Mk) is detected using collagen-based MegaCult™-C medium

A variety of resources are available on our website, including colony identification tutorials and an instructional video for setting up the CFU assay.

Visit www.stemcell.com/humancfuwallchart to learn more.



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