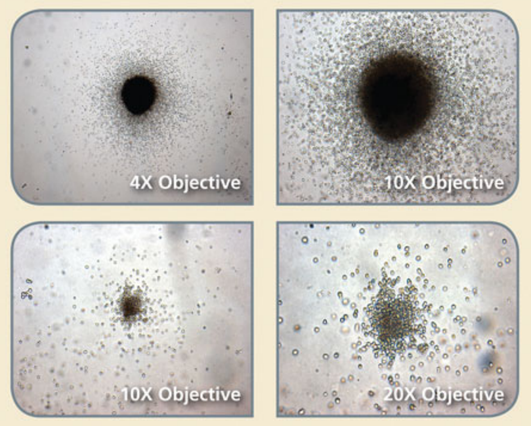
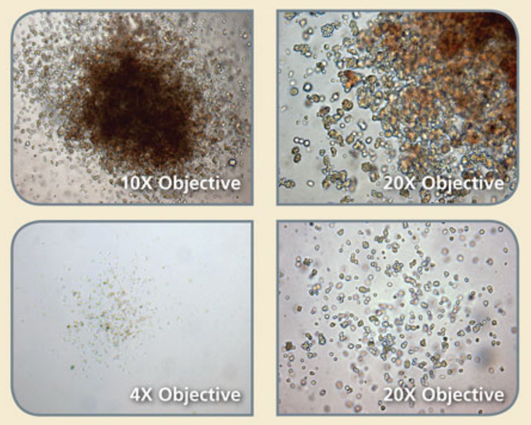
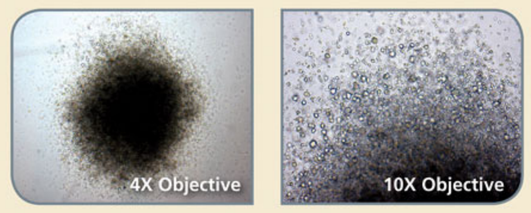
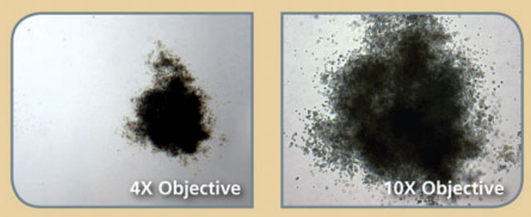
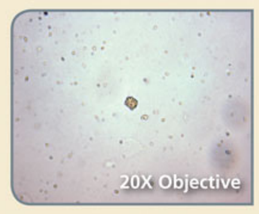
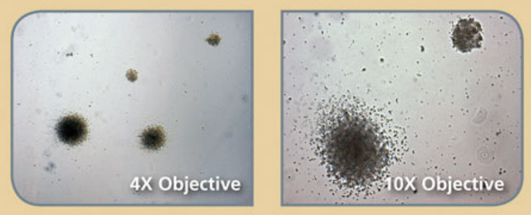
STEMCELL TECHNOLOGIES

A Guide to the Identification of Colonies Derived from Mouse Hematopoietic Progenitors

MEDIUM	FORMULATION	PROGENITORS DETECTED & DESCRIPTION	COLONIES
MethoCult® GF M3434 **MethoCult® GF M3534**	• Methylcellulose in Iscove's MDM • Fetal Bovine Serum • Bovine Serum Albumin • rh Insulin • Human Transferrin (Iron-Saturated) • 2-Mercaptoethanol • rm Stem Cell Factor • rm IL-3 • rh IL-6 • rh Erythropoietin (M3434 only) • Supplements	**CFU-GM** (Colony-Forming Unit-Granulocyte, Macrophage) *M3434 and M3534* • Cell membranes are distinguishable throughout the colony, permitting the identification of individual cells. • Cells tend to be "phase-bright" or "clear" in appearance. • Cells within the colony may be of different sizes. • In some colonies, cells can cluster together, forming a dense core that appears dark.	
		BFU-E (Burst-Forming Unit-Erythroid) *M3434 Only* • Erythroid cells tend to cluster together. These clusters resemble a bag of marbles or cauliflower. They can appear "wrinkled" and cells cannot be individually distinguished. For an example of a single cluster, see the CFU-E-derived colony below. • Macrophages can sometimes be observed in colonies derived from erythroid progenitors. • Cells in colonies derived from erythroid progenitors may appear hemoglobinized. • If the colony is large and the cells are clustered together in the center, look at the cells at the edge of the colony to determine if they have erythroid morphology.	
		**CFU-GEMM** (Colony-Forming Unit-Granulocyte, Erythroid, Macrophage, Megakaryocyte) *M3434 Only* • Colonies derived from these immature progenitors are generally very large. • Erythroid cells, where the clusters of cells appear "wrinkled" and cells cannot be individually distinguished, are present. • Most of the colony is composed of non-erythroid cells, which can be clearly distinguished and are "phase-bright." • The colony is likely to be large with cells concentrated in the middle so it is important to look at the cells at the edge of the colony to determine its lineage. • Megakaryocytes are often visible in colonies derived from CFU-GEMM. Megakaryocytes are very large cells (two to four times the size of macrophages) and have a glossy appearance, resembling soapy bubbles.	
MethoCult® SF M3436	• Methylcellulose in Iscove's MDM • Bovine Serum Albumin • rh Insulin • Human Transferrin (Iron-Saturated) • 2-Mercaptoethanol • Cytokines (including rh Erythropoietin) • Supplements *NOTE: This formulation does not support significant growth of non-erythroid progenitors*	**BFU-E** • Colonies derived from immature BFU-E are very large. • Cells often do not fully mature and therefore do not have the typical erythroid morphology, but they do hemoglobinize, giving the colony a red or brown appearance. • Mature BFU-E-derived colonies have a similar morphology to small BFU-E-derived colonies in M3434.	
		Non-BFU-E • Some colonies contain cells that do not display an erythroid morphology; they are scored as non-BFU-E-derived colonies.	
MethoCult® M3334	• Methylcellulose in Iscove's MDM • Fetal Bovine Serum • Bovine Serum Albumin • rh Insulin • Human Transferrin (Iron-Saturated) • 2-Mercaptoethanol • rh Erythropoietin • Supplements	**CFU-E** (Colony-Forming Unit-Erythroid) • Colonies derived from CFU-E are made up of a very small cluster containing at least six erythroblasts. These clusters resemble a bag of marbles or cauliflower. They can appear "wrinkled" and cells cannot be individually distinguished. • Clusters appear "rosy" in color and "glossy" in appearance.	
MethoCult® M3630	• Methylcellulose in Iscove's MDM • Fetal Bovine Serum • 2-Mercaptoethanol • rh IL-7 • Supplements	**Pre-B** • Cells within the colony are very small with a clear appearance. • The shape of the colony is typically a "starburst," but colonies can also have clean edges. • All cells within the colony are the same size. • Large clusters of macrophages may be present in the dish. They can be differentiated from pre-B-derived colonies based on cell size; macrophages are large.	
MethoCult® M3234	• Methylcellulose in Iscove's MDM • Fetal Bovine Serum • Bovine Serum Albumin • rh Insulin • Human Transferrin (Iron-Saturated) • 2-Mercaptoethanol • Supplements	**Progenitors detected depend on cytokines added** • Add GM-CSF to analyze CFU-GM, CFU-G and CFU-M • Add M-CSF to analyze CFU-M *Requires addition of cytokines as desired*	

Recommended Plating Concentrations and Incubation Times

PROGENITOR	METHOCULT® FORMULATION	TISSUE	PLATING CONCENTRATION (CELLS PER 35 MM DISH)	INCUBATION TIME
BFU-E, CFU-GM, CFU-GEMM	MethoCult® GF M3434	Bone Marrow	$2 - 3 \times 10^4$	10 - 12 days
		Spleen	$1 - 3 \times 10^5$	
		Peripheral Blood	$1 - 3 \times 10^5$	
BFU-E	MethoCult® SF M3436	Bone Marrow	$3 - 8 \times 10^4$	10 - 14 days
		Spleen	$1.5 - 5 \times 10^5$	
CFU-E	MethoCult® M3334	Bone Marrow	1×10^5	48 hours
		Spleen	$2 - 4 \times 10^5$	
Pre-B	MethoCult® M3630	Bone Marrow	$1 - 2 \times 10^5$	7 days
		Spleen	*Pre-B not present in spleen*	

Plating concentrations are shown for C57BL/6 and BALB/c mice. Plating concentration can vary with other strains or genetic modification.

Other Equipment Required to Perform Colony Assays

PRODUCT DESCRIPTION	CATALOG #	QUANTITY	APPLICATIONS
Iscove's Modified Dulbecco's Medium (IMDM) with 2% Fetal Bovine Serum	07700	100 mL	Washing hematopoietic cells.
Blunt-end needles	28110	100	Aliquoting MethoCult® and plating cultures. Recommended for prevention of needle-stick injuries.
	28120	2000	
3 cc syringe	28230	30	Aliquoting MethoCult® and plating cultures. Syringes and blunt-end needles are recommended when dispensing MethoCult®. The semi-solid medium will stick to the inside of a standard pipette, resulting in a less accurate volume.
	28240	100	
35 mm dishes for culture in MethoCult®	27100	10	Optimal colony growth without supporting adherent cells.
	27150	500	
60 mm gridded scoring dishes	27500	5	A 35 mm culture dish fits inside the 60 mm gridded scoring dish, which is a standard size for most microscope stages. 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, stage holder for a 60 mm gridded dish and a blue filter is required. A differential counter allows colonies of different lineages to be counted at the same time.			