

SmartDish™ and STEMgrid™-6

Meniscus-Free Cultureware for More Accurate Counting of Hematopoietic Colonies

Introduction

The colony-forming unit (CFU) assay is an *in vitro* functional assay for enumerating multipotential and lineage-committed hematopoietic progenitor cells (HPCs) in bone marrow, blood and other hematopoietic tissues. This assay has numerous applications for basic and clinical research in hematopoiesis, cord blood banking and hematopoietic stem cell transplantation. The assay is performed by culturing hematopoietic cells (depleted of any residual red blood cells) in MethoCult™ medium and counting the number of colonies produced by different sub-types of CFUs 7 to 14 days later. Hematopoietic colonies can be counted either manually using an inverted microscope or automatically using STEMvision™.

Preventing the Meniscus Effect

When a CFU assay is performed in traditional cultureware, a meniscus is formed between the culture medium and the sides of the dish. The meniscus results in an increased medium depth at the periphery of the dish, leading to a higher proportion of colonies forming along its edges (Figure 1A). Optical distortion at the edges can make colony identification challenging (Figures 2A and 2C), reducing accuracy through undercounting of CFUs.

SmartDish™ 6-well culture plates (Catalog #27370) have been designed to enable accurate and reproducible colony counting by preventing the formation of a meniscus. This allows for an even distribution of culture medium, resulting in a more uniform distribution of colonies throughout the entire well (Figure 1B). The absence of a meniscus also reduces optical distortion so that colonies located at the edge can be more easily counted (Figures 2B and 2D).

SmartDish™ has been designed to work with standard inverted microscopes when performing manual colony counts (Figure 2B), as well as with the STEMvision™ instrument (Catalog #22000) for automated counting of hematopoietic CFU assays (Figure 2D). For manual counting of hematopoietic CFU assays, SmartDish™ is used with STEMgrid™-6 (Catalog #27000), a detachable counting grid that facilitates navigation throughout the culture, dividing it into four quadrants for partial counting, if desired.

Why Use SmartDish™ ?

CONSISTENT. Results in an even distribution of colonies throughout each well.

CLEAR. No shadow or optical distortion at well edges.

ACCURATE. Colonies in SmartDish™ plates may be counted using automated methods.

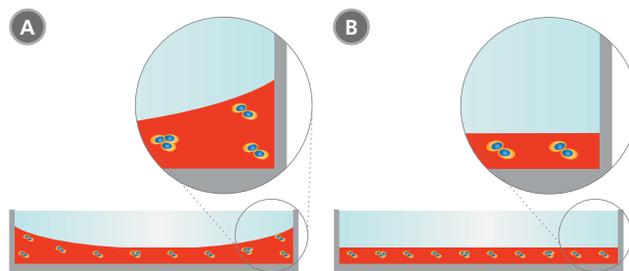
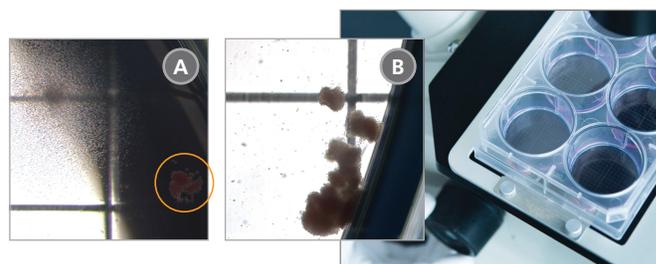


Figure 1. Schematic Illustration of Medium and Colony Distribution in Standard and SmartDish™ Cultureware

The formation of a meniscus in (A) standard cultureware results in more colonies forming the periphery of the dish where the culture medium is deeper. The absence of a meniscus in (B) SmartDish™ cultureware ensures more uniform distribution of culture medium and colonies throughout the entire well.

Manual Counting of CFU Assays



Automated Counting of CFU Assays

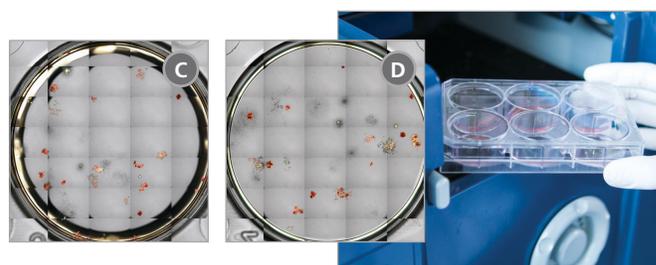


Figure 2. CFU Assays Performed in Standard or SmartDish™ 6-Well Plates

Shown are representative 35 mm wells from (A,C) standard and (B,D) SmartDish™ culture plates, counted (A,B) manually with STEMgrid™-6 attached or (C,D) using STEMvision™. Optical distortion caused by the meniscus obscures colonies at the edge of cultures grown in standard plates (A, C) and makes them more difficult to count (see circled colony in A). Colonies are more easily seen and counted at the edge of the SmartDish™ wells which have been treated to eliminate meniscus formation (B, D).

SmartDish™ and STEMgrid™-6

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SmartDish™ and STEMgrid™-6 Product Information

PRODUCT	CATALOG #	FORMAT
SmartDish™ 6-Well Plates	27370 27371	5 / pack 50 / pack
STEMgrid™-6 Counting Grid	27000	1 / pack



SmartDish™



STEMgrid™-6

Popular MethoCult™ Media for Human Cells

METHOCULT™ PRODUCT	CATALOG #	SIZE	COMPONENTS				GROWTH FACTORS	APPLICATIONS/DESCRIPTION	
			MC	FBS	BSA	2-ME			
MethoCult™ Optimum*	04034** 04044**	100 mL 24 x 3 mL	✓	✓	✓	✓	rhSCF, rhG-CSF, rhGM-CSF, rhIL-3, rhEPO	<ul style="list-style-type: none"> Assays of human clonogenic HPCs from CB, BM, PB, and leukapheresis products With rhEPO: Support growth of CFU-E, BFU-E, CFU-G, CFU-M, CFU-GM and CFU-GEMM Without rhEPO: Support growth of CFU-G, CFU-M, and CFU-GM 	<ul style="list-style-type: none"> Optimal formulation The presence of G-CSF improves the discrimination of myeloid colony subtypes
MethoCult™ Optimum without EPO*	04035** 04045**	100 mL 24 x 3 mL	✓	✓	✓	✓	rhSCF, rhG-CSF, rhGM-CSF, rhIL-3, no rhEPO		<ul style="list-style-type: none"> Original formulation used by labs wanting to keep historical protocols
MethoCult™ Classic	04434 04444	100 mL 24 x 3 mL	✓	✓	✓	✓	rhSCF, rhGM-CSF, rhIL-3, rhEPO		<ul style="list-style-type: none"> For use with CD34+ cells and cells isolated by other purification methods
MethoCult™ Classic without EPO	04534 04544	100 mL 24 x 3 mL	✓	✓	✓	✓	rhSCF, rhGM-CSF, rhIL-3, no rhEPO		<ul style="list-style-type: none"> CFU assays performed as part of an LTC-IC assay
MethoCult™ Enriched	04435** 04445**	100 mL 24 x 3 mL	✓	✓	✓	✓	rhSCF, rhG-CSF, rhGM-CSF, rhIL-3, rhIL-6, rhEPO		
MethoCult™ Enriched without EPO	04535** 04545**	100 mL 24 x 3 mL	✓	✓	✓	✓	rhSCF, rhG-CSF, rhGM-CSF, rhIL-3, rhIL-6, no rhEPO		

MC: Methylcellulose; FBS: Fetal Bovine Serum; BSA: Bovine Serum Albumin; 2-ME: 2-Mercaptoethanol; EPO: Erythropoietin; CB: Cord Blood; BM: Bone Marrow; PB: Peripheral Blood; HPCs: Hematopoietic Progenitor Cells; rh: Recombinant Human; LTC-IC: Long-Term Culture-Initiating Cell

* Validated for automated counting of hematopoietic CFU assays using STEMvision™

** CE-marked formulations also available for in vitro diagnostic (IVD) use in select regions, visit www.stemcell.com/regulated-products for more information.

Popular MethoCult™ Media for Mouse Cells

METHOCULT™ PRODUCT	CATALOG #	SIZE	COMPONENTS					GROWTH FACTORS	APPLICATIONS/DESCRIPTION	
			MC	FBS	BSA	Insulin + Transferrin	2-ME			
MethoCult™ GF M3434*	03434 03444	100 mL 24 x 3 mL	✓	✓	✓	✓	✓	rhEPO, rmSCF, rhIL-6, rmIL-3	Assays of mouse hematopoietic progenitors (BFU-E, CFU-G, CFU-M, CFU-GM, CFU-GEMM) in BM, spleen, PB and fetal liver	
MethoCult™ GF M3534*	03534	100 mL	✓	✓	✓	✓	✓	rmSCF, rhIL-6, rmIL-3, no rhEPO	Assays of mouse myeloid progenitor (CFU-G, CFU-M, CFU-GM) derived colonies from BM, spleen, PB and fetal liver	
MethoCult™ SF M3436*	03436	100 mL	✓	✓	✓	✓	✓	Cytokines (including rh EPO)	Assays of mouse erythroid progenitor (BFU-E) derived colonies from BM and other tissues	
MethoCult™ M3234	03234	80 mL	✓	✓	✓	✓	✓	no rhEPO, no additional cytokines	Base medium for CFU assays, allowing addition of growth factors of choice	

MC: Methylcellulose; FBS: Fetal Bovine Serum; BSA: Bovine Serum Albumin; 2-ME: 2-Mercaptoethanol; EPO: Erythropoietin; BM: Bone Marrow; PB: Peripheral Blood; rh: Recombinant Human, rm: Recombinant Mouse.

*Validated for automated counting of hematopoietic CFU assays using STEMvision™

For related products, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com/HSPCworkflow or contact us at techsupport@stemcell.com. For available fresh and cryopreserved peripheral blood, cord blood and bone marrow products in your region, visit www.stemcell.com/primarycells.

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