

**PRODUCT DESCRIPTION**

The colony forming cell (CFC) assay is an *in vitro* quantitative assay used in the study of hematopoietic stem cells. The assay is based on the ability of hematopoietic progenitors to proliferate and differentiate into colonies in a semi-solid media in response to cytokine stimulation. The colonies formed can be enumerated and characterized according to their unique morphology.

Human Methylcellulose Complete Media without Epo is specially formulated and has been optimized for CFC assays using colony-forming myeloid progenitors (CFU-GM, CFU-G, CFU-M) of human origin. This product can also be used in the long-term culture-initiating cell (LTC IC) assay.

**STABILITY & STORAGE**

Upon receipt, the Methylcellulose Complete Media without Epo and Cell Resuspension Solution should be stored at ≤ -20 °C in a manual defrost freezer. The media can be thawed at 2-8 °C overnight. Thawed supplement can be aliquoted and stored at ≤ -20 °C in a manual defrost freezer until expiration date.

**PRECAUTION**

The acute and chronic effects of overexposure to this media are unknown. Safe laboratory procedures should be followed and protective clothing should be worn when handling this media.

**REAGENTS PROVIDED**

	CONTENTS	CONCENTRATION
<b>Human Methylcellulose Complete Media without Epo</b> (Part # 390396) 100 mL	Methylcellulose (1500 cps) in Iscove's Modified Dulbecco's Media	1.4%
	Fetal Bovine Serum	25%
	Bovine Serum Albumin	2%
	L-Glutamine	2 mM
	2-Mercaptoethanol	5 x 10 <sup>-5</sup> M
	Recombinant Human SCF	50 ng/mL
	Recombinant Human GM-CSF	10 ng/mL
	Recombinant Human IL-3	10 ng/mL

	CONTENTS	CONCENTRATION
<b>Cell Resuspension Solution</b> (Part # 390397) 15 mL	Fetal Bovine Serum in Iscove's Modified Dulbecco's Media	50%

## REAGENT & MATERIAL PREPARATION

### Thawing and Aliquotting the Methylcellulose Complete Media

1. Thaw the bottle of media at 2-8 °C overnight. Do not shake the bottle if ice is still present.
2. After complete thawing, shake the bottle vigorously to thoroughly mix the contents. Air bubbles will form due to the vigorous mixing procedure.
3. Allow the air bubbles to escape by placing the bottle either at room temperature or at 2-8 °C for 30-60 minutes.
4. Use a sterile laboratory pipetting needle attached to a 10 mL syringe. Dispense the exact amount of media required into sterile 5 mL vials. The table below serves as a guide for aliquotting the product.

For experiments using cell samples in:	
Duplicate	Triplicate
3 mL	4 mL

### Thawing and Aliquotting Cell Resuspension Solution

1. Thaw the bottle at 2-8 °C.
2. Mix the solution thoroughly using a serological pipette.
3. Aliquot and store at ≤ -20° C in a manual defrost freezer. Do not use past the expiration date.

### Thawing Aliquots

1. Just before use, bring the vials of Methylcellulose Complete Media without Epo and Cell Resuspension Solution to room temperature and thaw without disturbance.

## PROCEDURE

The protocol for a CFC assay varies depending upon the practice of each laboratory. A sample protocol for setting up the Methylcellulose Assay is available at [www.RnDSystems.com/go/HumanMethylcelluloseProtocol](http://www.RnDSystems.com/go/HumanMethylcelluloseProtocol).

The table below provides the recommended volume of cells and supplements/cytokines to be added to the Methylcellulose Complete Media without Epo for cell plating. The methylcellulose concentration in the final cell mixture should be 1.27%.

Catalog Number	For experiments using cell samples in	
	Duplicate	Triplicate
HSC004	3 mL	4 mL
Supplement/Cytokine	N/A	N/A
Cells	0.3 mL	0.4 mL

## LIMITATIONS OF PROCEDURE

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- This reagent should not be used beyond the expiration date indicated on the label.
- The media is optimized to assay human hematopoietic progenitors and is ineffective with mouse hematopoietic progenitors.
- Results may vary due to variations between human hematopoietic progenitors derived from different individuals.

## TECHNICAL HINTS

- The 5 mL polypropylene vials are recommended since they are compatible with most laboratory syringes and can accommodate effective mixing of the viscous methylcellulose media with cells and other culture components
- Due to the high viscosity of the methylcellulose media, use of a syringe is necessary to accurately measure the media volume.
- The laboratory pipetting needle from Popper & Sons (Catalog # 7941) or Thermo Fisher Scientific (Catalog # 14-825-16M) is recommended for aliquotting the Methylcellulose Concentrate due to the large diameter. The pipetting needle can be autoclaved and reused.