



Human Methylcellulose Base Media

Catalog Number: HSC002

Storage: $\leq -20^{\circ}\text{C}$

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 medium in response to cytokine stimulation. The colonies formed can be enumerated and characterized according to their unique morphology.

The Human Methylcellulose Base Media contains components that have been optimized for CFC assays. Individual researchers can customize the media by adding cells and other culture supplements tailored to their specific research. This product can also be used in the long-term culture-initiating cell (LTC-IC) assay.

Reagents Provided

1. Human Methylcellulose Base Media (Part # 390394)

90 mL

Contents	Concentration (when diluted to a final volume of 100 mL)
Methylcellulose (1500 cps) in Iscove's Modified Dulbecco's Medium	1.4%
Fetal Bovine Serum	25%
Bovine Serum Albumin	2%
L-Glutamine	2 mM
2-Mercaptoethanol	$5 \times 10^{-5}\text{ M}$

2. Cell Resuspension Solution (Part # 390397)

15 mL

Contents	Concentration
Fetal Bovine Serum in Iscove's Modified Dulbecco's Medium	50%

Reagent Storage and Handling

Sterile technique is required when handling these reagents.

I. Storage

- The Methylcellulose Base Media and Cell Resuspension Solution should be stored at $\leq -20^{\circ}\text{C}$ upon receipt. Storage at $2 - 8^{\circ}\text{C}$ is not recommended.

II. Thawing and Aliquotting of Methylcellulose Base Media

- Thaw the bottle of media at $2 - 8^{\circ}\text{C}$ overnight. Do not shake the bottle if ice is still present.
- After complete thawing, shake the bottle vigorously to thoroughly mix the contents. Air bubbles will form due to the vigorous mixing procedure.
- Allow the air bubbles to escape by placing the bottle either at room temperature or at $2 - 8^{\circ}\text{C}$ for 30 - 60 minutes.
- 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.

Catalog Number	For experiments using cell samples in	
	Duplicate	Triplicate
HSC002	2.7 mL	3.6 mL

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- ◆ The 5 mL vials from R&D Systems (Catalog # HSC999) 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 aliquoting the methylcellulose media due to its large diameter. The pipetting needle can be autoclaved and reused.

E. Store aliquots at $\leq -20^{\circ}\text{C}$ in a manual defrost freezer until use. Do not use past the expiration date.

III. Thawing and Aliquotting Cell Resuspension Solution

- A. Thaw the bottle at $2 - 8^{\circ}\text{C}$.
- B. Mix the solution thoroughly using a serological pipette.
- C. Aliquot and store at $\leq -20^{\circ}\text{C}$ in a manual defrost freezer. Do not use past the expiration date.

IV. Thawing Aliquots

- A. Just before use, bring the vials of Methylcellulose Base Media 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 <http://www.RnDSystems.com/go/HumanMethylcelluloseProtocol>.

The table below provides the recommended volume of cells and supplements/cytokines to be added to the Methylcellulose Base Media 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
HSC002	2.7 mL	3.6 mL
Supplement/Cytokine	0.3 mL	0.4 mL
Cells	0.3 mL	0.4 mL

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.

Limitations of the Procedure

- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- The reagents should not be used beyond the expiration date indicated on the vial labels.
- 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.