Human Methylcellulose Serum-Free Enriched Media

Catalog Number: HSC005SF
Storage: ≤ -20° C

Product Description

The colony forming cell (CFC) assay is an *in vitro* quantitative assay used in the study of hematopoietic progenitor 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.

The Human Methylcellulose Serum-Free Enriched Media is specially formulated and has been optimized for CFC assays, detecting burst-forming and colony-forming erythroid (BFU-E, CFU-E), myeloid (CFU-GM, CFU-G, CFU-M), and mixed lineage (CFU-GEMM) progenitors in human bone marrow, cord blood, mobilized peripheral blood, and peripheral blood samples. It can also be used in CFC assays for purified human hematopoietic progenitor cells. This product is recommended for use in the CFC assays, when a serum-free defined condition is desired.

Reagents Provided

Human Methylcellulose Serum-Free Enriched Media (Part # 390518) **100 mL**

Contents	Concentration
Methylcellulose (1500 cps) in Iscove's Modified Dulbecco's Media	1.4%
Human Transferrin	300 µg/mL
Bovine Serum Albumin	3.6%
Recombinant Human Insulin	16 µg/mL
L-Glutamine	2 mM
2-Mercaptoethanol	5×10^{-5} M
Recombinant Human SCF	50 ng/mL
Recombinant Human GM-CSF	20 ng/mL
Recombinant Human G-CSF	20 ng/mL
Recombinant Human IL-3	20 ng/mL
Recombinant Human IL-6	20 ng/mL
Recombinant Human Epo	3 IU/mL
Cholesterol	Trace

Reagent Storage and Handling

Sterile technique is required when handling these reagents.

I. Storage
   A. The Human Methylcellulose Serum-Free Enriched Media should be stored at ≤ -20° C upon receipt. Storage at 2 - 8° C is not recommended.

II. Thawing and Aliquotting Human Methylcellulose Serum-Free Enriched Media
   A. Thaw the bottle of media at 2 - 8° C overnight. Do not shake the bottle if ice is still present.
   B. After complete thawing, shake the bottle vigorously to thoroughly mix the contents. Air bubbles will form due to the vigorous mixing procedure.
   C. Allow the air bubbles to escape by placing the bottle either at room temperature or at 2 - 8° C for 30 - 60 minutes.

D. 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**
3.0 mL	4.0 mL

- 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 aliquotting the methylcellulose media due to its large diameter. The pipetting needle can be autoclaved and reused.

E. Store the aliquots at ≤ -20° C in a manual defrost freezer until use. Do not use past the expiration date.

III. Thawing Aliquots

A. Just before use, bring the vials of Human Methylcellulose Serum-Free Enriched Media 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 Human Methylcellulose Serum-Free Enriched Media for cell plating. The cells can be resuspended in IMDM or the appropriate media to the desired cell concentration for plating. The methylcellulose concentration in the final cell mixture should be 1.27%.

	For experiments using cell samples in	
	Duplicate	**Triplicate**
Catalog # HSC005SF	3.0 mL	4.0 mL
Supplement/Cytokine	None Needed	None Needed
Cells	0.3 mL	0.4 mL

Precautions

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

The human Transferrin used in this product was derived from human plasma, which has been tested and found negative for HIV-1/2 antibodies, Hepatitis B surface antigen, Hepatitis C antibody, Syphilis, and p24 antigen by FDA approved methods. Handle as if capable of transmitting infection, and dispose of according to applicable regulations.

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.