Mouse Methylcellulose Complete Media for Pre-B Cells

Catalog Number: HSC009
Storage: ≤ -20° 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 Mouse Methylcellulose Complete Media for Pre-B cells is specially formulated and has been optimized for CFC assays to identify Pre-B progenitor cells of mouse origin.

Reagents Provided

Mouse Methylcellulose Complete Media for Pre-B Cells — **100 mL**

Contents	Concentration
Methylcellulose (1500 cps) in Iscove's Modified Dulbecco's Medium	1.3%
Fetal Bovine Serum	20%
L-Glutamine	2 mM
2-Mercaptoethanol	5×10^{-5} M
Recombinant Mouse IL-7	10 ng/mL

Reagent Storage and Handling

Sterile technique is required when handling these reagents.

I. Storage
   A. The Mouse Methylcellulose Complete Media for Pre-B Cells should be stored at ≤ -20° C upon receipt. Storage at 2 - 8° C is not recommended.

II. Thawing and Aliquotting of Mouse Methylcellulose Complete Media for Pre-B Cells
   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.

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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.

Catalog Number	For experiments using cell samples in	
	Duplicate	Triplicate
HSC009	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.
- A laboratory pipetting needle from Popper & Sons (Catalog # 7491) or Thermo Fisher Scientific (Catalog # 14-825-16M) is recommended for aliquoting the methylcellulose media due to its larger diameter. The pipetting needle can be autoclaved and reused.

E. Store 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 Mouse Methylcellulose Complete Media for Pre-B Cells 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 Mouse Methylcellulose Assay is available at **http://www.RnDSystems.com/go/Pre-BProtocol.**

The table below provides the recommended volume of cells and supplements/cytokines to be added to the Mouse Methylcellulose Complete Media for Pre-B Cells for cell plating. The methylcellulose concentration in the final cell mixture should be 1.17%.

Catalog Number	For experiments using cell samples in	
	Duplicate	Triplicate
HSC009	3.0 mL	4.0 mL
Cells	0.3 mL	0.4 mL
Supplement/Cytokine	None Needed	None Needed

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 have not been established.
- The reagents should not be used beyond the expiration date indicated on the vial labels.
- The media is optimized to assay mouse hematopoietic progenitors and is ineffective with human hematopoietic progenitors.
- Results may vary due to variations between mouse hematopoietic progenitors.