Rat Methylcellulose Complete Media without Epo

Catalog Number: HSC012
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.

Rat 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 rat origin. This product can also be used in the long-term culture-initiating cell (LTC-IC) assay.

Reagents Provided

1. Rat Methylcellulose Complete Media without Epo (Part # 390531) **100 mL**

Contents	Concentration
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^{-5} M
Recombinant Rat SCF	50 ng/mL
Recombinant Rat GM-CSF	10 ng/mL
Recombinant Rat IL-3	10 ng/mL

Reagent Storage and Handling

Sterile technique is required when handling these reagents.

I. Storage
   A. The Methylcellulose Complete Media without Epo should be stored at ≤ -20 °C upon receipt. Storage at 2-8 °C is not recommended.

II. Thawing and Aliquotting Methylcellulose Complete Media without Epo
   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 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 Methylcellulose Complete Media without Epo 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/MouseMethylcelluloseProtocol.***

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%.

	For experiments using cell samples in	
Catalog Number	**Duplicate**	**Triplicate**
HSC012	3.0 mL	4.0 mL
Supplement/Cytokine	None Needed	None Needed
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.
- This reagent should not be used beyond the expiration date indicated on the vial label.
- The media is optimized to assay rat hematopoietic progenitors and is ineffective with human and mouse hematopoietic progenitors.
- Results may vary due to variations between rat hematopoietic progenitors derived from different animals.