MethoCult™ M3334

Methylcellulose-Based Medium with EPO (Without Other Cytokines) for Mouse Cells

Catalog #03334 90 mL

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

Methylcellulose Medium for Colony-Forming Unit (CFU) Assays for Mouse Cells

MethoCult™ M3334 is suitable for the detection and quantification of hematopoietic progenitor cells in mouse bone marrow (BM) using CFU assays.

MethoCult™ M3334 has been formulated to support optimal growth of mature mouse erythroid progenitor cells (CFU-E and mature BFU-E).

For the detection of granulocyte-macrophage progenitor cells (CFU-GM, CFU-G, CFU-M) and multipotential granulocyte, erythroid, macrophage, megakaryocyte progenitor cells (CFU-GEMM), additional growth factors are required.

Properties

Storage: Store at -20°C.

Shelf Life: Stable until expiry date (EXP) on label.

Contains:

- Methylcellulose in Iscove's MDM
- Fetal bovine serum
- Bovine serum albumin
- Recombinant human insulin
- Human transferrin (iron-saturated)
- 2-Mercaptoethanol
- Recombinant human erythropoietin (EPO)
- Supplements

This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Handling / Directions For Use

NOTE: If product is received partially thawed, place immediately at -20°C or thaw and aliquot as described below.

PREPARATION OF COMPLETE METHOCULT™ M3334 MEDIUM

MethoCult™ M334 does not contain cytokines other than EPO. Cytokines can be added directly to the bottle or to each tube after aliquoting. Refer to Table 1 for volumes required to prepare complete MethoCult™ M3334 medium per bottle or per tube. The 9:1 (v:v) ratio of MethoCult™ to other components in the liquid medium (e.g. cytokines) gives the correct viscosity for optimal CFU growth and morphology.

Use sterile techniques to prepare complete MethoCult™ M3334 medium (MethoCult™ M3334 base medium + desired components).

NOTE: Do not use pipettes to dispense methylcellulose as the volume dispensed will not be accurate. Syringes and large-bore blunt-end needles should be used for accurate dispensing of viscous methylcellulose medium and to prevent needle-stick injuries.

A. TO PREPARE 100 mL BOTTLE

1. Thaw 90 mL bottle of MethoCult™ M3334 at room temperature (15 - 25°C) or overnight at 2 - 8°C. Do not thaw MethoCult™ at 37°C.
2. Prepare desired growth factors, supplements, and Iscove's Modified Dulbecco's Medium (IMDM; Catalog #36150) in a volume of 10 mL and add to MethoCult™ (total volume of 100 mL). Shake vigorously for 1 - 2 minutes and then let stand for at least 5 minutes to allow bubbles to rise to the top before aliquoting.
3. Using a 3 or 6 mL luer lock syringe attached to a 16 gauge Blunt-End Needle (Catalog #28110), aliquot 3 mL per tube for 1.1 mL duplicate cultures or 4 mL per tube for 1.1 mL triplicate cultures. Complete MethoCult™ medium is now ready for use.

B. TO PREPARE INDIVIDUAL TUBES

1. Thaw 90 mL bottle of MethoCult™ M3334 at room temperature (15 - 25°C) or overnight at 2 - 8°C. Do not thaw MethoCult™ at 37°C.
2. Shake vigorously for 1 - 2 minutes and then let stand for at least 5 minutes to allow bubbles to rise to the top before aliquoting.
3. Using a 3 or 6 mL luer lock syringe attached to a 16 gauge Blunt-End Needle (Catalog #28110), aliquot MethoCult™ M3334 base medium into tubes (see Table 1 for required volumes).

   NOTE: Before adding components, tubes of incomplete MethoCult™ medium may be stored at -20°C until expiry date as indicated on label. After thawing aliquoted tubes, add desired components and mix well.
4. Add desired growth factors, supplements, and Iscove's Modified Dulbecco's Medium (IMDM; Catalog #36150) to tubes of MethoCult™ M3334 (see Table 1 for required volumes).
5. Vortex tubes to mix well. Complete MethoCult™ medium is now ready for use.
6. Aliquot any remaining MethoCult™ M3334 base medium for duplicate or triplicate cultures (see Table 1 for required volumes), store at -20°C, then add desired components after thawing. Mix well before use.

Table 1. Volumes Required for Preparation of Complete MethoCult™ M3334 Medium

COMPONENT	PER BOTTLE	PER TUBE (**duplicate** 1.1 mL cultures)	PER TUBE (**triplicate** 1.1 mL cultures)
MethoCult™ M3334	90 mL	2.7 mL	3.6 mL
IMDM with cytokines*	10 mL	0.3 mL	0.4 mL
TOTAL VOLUME	100 mL	3.0 mL	4.0 mL

*For a complete list of available cytokines, refer to our website at www.stemcell.com.

For recommended cell plating concentrations, setup of mouse CFU assays, and counting and classification of mouse colonies, refer to the Technical Manual: Mouse Colony-Forming Unit Assays Using MethoCult™ (Document #28405), available on our website at www.stemcell.com or contact us to request a copy.

References

Miller CL & Lai B. (2005) Human and mouse hematopoietic colony-forming cell assays. In: Helgason CD & Miller CL (Eds.). Basic Cell Culture Protocols (pp. 71–89). Totowa, New Jersey: Humana Press Inc.