MethoCult™ M3334

Methylcellulose-based medium with EPO (without other cytokines) for mouse cells

Catalog #03334 90 mL

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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 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, and CFU-M) and multipotential granulocyte, erythroid, macrophage, megakaryocyte progenitor cells (CFU-GEMM), additional growth factors are required.

Properties

Storage: Store at -20°C.

NOTE: Product may be shipped with dry ice or ice packs and may be received thawed.

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 technique 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 MethoCult™ M3334 at room temperature (15 - 25°C) or overnight at 2 - 8°C. Do not thaw at 37°C.
2. Prepare desired growth factors, supplements, and Iscove's Modified Dulbecco's Medium (IMDM; Catalog #36150) in 10 mL and add to the 90 mL bottle of 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 luer lock syringe (3 mL [Catalog #28230] or 6 mL) attached to a 16 Gauge Blunt-End Needle (Catalog #28110), aliquot as follows:
   - 3 mL per tube for 1.1 mL duplicate cultures
   - 4 mL per tube for 1.1 mL triplicate cultures

B. TO PREPARE INDIVIDUAL TUBES

1. Thaw MethoCult™ M3334 at room temperature (15 - 25°C) or overnight at 2 - 8°C. Do not thaw 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 luer lock syringe (3 mL [Catalog #28230] or 6 mL) 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 the 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 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 (CFU) Assays Using MethoCult™ (Document #10000005597), available at www.stemcell.com, or contact us to request a copy.

Related Products

For related products, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com/HSPCworkflow, or contact us at techsupport@stemcell.com. For available fresh and cryopreserved peripheral blood, cord blood, and bone marrow products, visit www.stemcell.com/primarycells.

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.