

MethoCult™ M3231

Methylcellulose-based medium without cytokines for mouse cells

Catalog #03231

80 mL



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Product Description

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

MethoCult™ M3231 is recommended as a base for the preparation of methylcellulose-based medium for the culture of mouse progenitor cells.

This incomplete medium formulation contains components that have been selected for optimal growth of mouse hematopoietic CFU. It is suitable for growth and detection of erythroid progenitor cells (BFU-E), granulocyte-macrophage progenitor cells (CFU-GM, CFU-G, and CFU-M), and multipotential granulocyte, erythroid, macrophage, megakaryocyte progenitor cells (CFU-GEMM) with the addition of appropriate growth factors and supplements. MethoCult™ M3231 can be used for the detection and quantification of hematopoietic progenitor cells in mouse bone marrow, spleen, peripheral blood, and fetal liver samples using CFU assays. This formulation does not contain erythropoietin (EPO) or other cytokines.

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
- 2-Mercaptoethanol
- Supplements

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™ M3231 MEDIUM

MethoCult™ M3231 base medium does not contain EPO or other cytokines. These can be added directly to the bottle or to each tube after aliquoting. Refer to Table 1 for volumes required to prepare complete MethoCult™ M3231 medium per bottle or per tube. The 4:1 (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™ M3231 medium (MethoCult™ M3231 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™ M3231 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 20 mL and add to the 80 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

Complete MethoCult™ medium is now ready for use.

B. TO PREPARE INDIVIDUAL TUBES

1. Thaw MethoCult™ M3231 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, aliquot MethoCult™ M3231 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™ M3231 (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™ M3231 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™ M3231 Medium

COMPONENT	PER BOTTLE	PER TUBE (duplicate 1.1 mL cultures)	PER TUBE (triplicate 1.1 mL cultures)
MethoCult™ M3231	80 mL	2.4 mL	3.2 mL
IMDM with cytokines*	20 mL	0.6 mL	0.8 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.

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