MethoCult™ SF H4236

Serum-free methylcellulose-based medium without cytokines for human cells

Catalog #04236 80 mL

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Product Description

Serum-Free Methylcellulose Medium for Colony-Forming Unit (CFU) Assays for Human Cells

MethoCult™ SF H4236 is recommended as a base medium for the culture of human cells in defined serum-free conditions, and to detect and quantify hematopoietic progenitor cells in human bone marrow (BM), mobilized peripheral blood (MPB), peripheral blood (PB), and cord blood (CB) samples using CFU assays. This formulation allows for the addition of an exogenous source of erythropoietin (EPO) and other cytokines, and is ideal for testing cytokine effects where the presence of fetal bovine serum is not desired.

Properties

Storage: Store at -20°C.

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

Contains:

- Methylcellulose in Iscove's MDM
- Bovine serum albumin
- Recombinant human insulin
- Human transferrin (iron-saturated)
- 2-Mercaptoethanol
- 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 requirements.

Handling / Directions for Use

NOTE: If product is received partially thawed, place immediately at -20°C or thaw and aliquot as described below. Do not use MethoCult™ past the expiry date as indicated on the label.

PREPARATION OF COMPLETE METHOCULT™ SF H4236 MEDIUM

MethoCult™ SF H4236 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™ SF H4236 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 to ensure optimal CFU growth and morphology.

Use sterile techniques to prepare complete MethoCult™ SF H4236 medium (MethoCult™ SF H4236 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 80 mL bottle of MethoCult™ SF H4236 at room temperature (15 - 25°C) or overnight at 2 - 8°C.
   NOTE: Do not thaw MethoCult™ at 37°C.
2. Prepare desired components in Iscove's Modified Dulbecco's Medium (IMDM; Catalog #36150) in 20 mL and add to MethoCult™ (total volume of 100 mL).
3. 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.
4. 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 80 mL bottle of MethoCult™ at room temperature (15 - 25°C) or overnight at 2 - 8°C.
   NOTE: 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™ SF H4236 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™ SF H4236 (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™ SF H4236 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™ SF H4236 Medium

COMPONENT	PER BOTTLE	PER TUBE (**duplicate** 1.1 mL cultures)	PER TUBE (**triplicate** 1.1 mL cultures)
MethoCult™ SF H4236	80 mL	2.4 mL	3.2 mL
IMDM with cytokines*	20 mL	0.6 mL	0.8 mL
TOTAL VOLUME	100 mL	3 mL	4 mL

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

For recommended cell plating concentrations, setup of human CFU assays, and counting and classification of human colonies, refer to the Technical Manual: Human Colony-Forming Unit Assays Using MethoCult™ (Document #10000005589), available at www.stemcell.com, or contact us to request a copy.

Notes and Tips

STEMCELL Technologies recommends the use of Human LDL (Catalog #02698) as a culture supplement. It has been prescreened for the culture, expansion, and colony assay of human hematopoietic and non-hematopoietic cells in serum-free culture media. It promotes the proliferation and survival of human hematopoietic and other progenitor cells in culture, resulting in increased cell output in expansion cultures and increased colony numbers and/or colony size in colony assays.

References

Atlas of Hematopoietic Colonies from Cord Blood. (2010). Vancouver: STEMCELL Technologies Inc. (Catalog #29940)

Eaves CJ & Eaves AC. (2006) Anatomy and physiology of hematopoiesis. In: Pui CH (Ed.). Childhood Leukemia, Second Edition (pp.69–105). Cambridge: Cambridge University Press.

Eaves C & Lambie K. (1995) Atlas of Human Hematopoietic Colonies. Vancouver: STEMCELL Technologies Inc. (Catalog #28700)

Nissen-Druey C et al. (2005) Human hematopoietic colonies in health and disease. Basel, Switzerland: S. Karger Medical and Scientific Publishers. (Catalog #28760)

Wognum B et al. (2013) Colony forming cell assays for human hematopoietic progenitor cells. In: Helgason CD & Miller CL (Eds.). Basic Cell Culture Protocols (pp. 267–83). Clifton, New Jersey: Humana Press Inc.