MethoCult™ H4330

Methylcellulose-Based Medium With EPO for Human Cells

Catalog #04330 90 mL

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Product Description

Colony-Forming Unit (CFU) Assays for Human Cells

MethoCult™ H4330 is recommended as a base medium 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 cytokines. It can also be used without the addition of exogenous cytokines for the assessment of colony-stimulating factor activity/burst-promoting activity in unknown samples.

Other formats of MethoCult™ H4330 medium, including tubes, are available as a custom order. Please contact info@stemcell.com for additional information.

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
- 2-Mercaptoethanol
- Recombinant human erythropoietin (EPO)
- 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™ H4330 MEDIUM

MethoCult™ H4330 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™ H4330 medium per bottle or per tube. The ratio (v:v) of MethoCult™ to other components in liquid medium (e.g. cytokines) is important for viscosity, which ensures optimal CFU growth and morphology.

Use sterile techniques to prepare complete MethoCult™ H4330 medium (MethoCult™ H4330 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™ H4330 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™ (for a 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™ H4330 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 2.7 mL per tube for 1.1 mL duplicate cultures or 3.6 mL per tube for 1.1 mL triplicate cultures.

   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 (see step 4) and mix well.
4. Add desired growth factors, supplements, and Iscove's Modified Dulbecco's Medium (IMDM; Catalog #36150) to tubes of MethoCult™ H4330 (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™ H4330 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™ H4330 Medium

COMPONENT	PER BOTTLE	PER TUBE (**duplicate** 1.1 mL cultures)	PER TUBE (**triplicate** 1.1 mL cultures)
MethoCult™ H4330	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 human CFU assays, and counting and classification of colonies, refer to the Technical Manual: Human Colony-Forming Cell Assays Using MethoCult™ (Document #28404), available on our website at www.stemcell.com, or contact us to request a copy.

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.