MethoCult™ H4100

Base methylcellulose medium for human cells

Catalog #04100 40 mL

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Product Description

Colony-Forming Unit (CFU) Assays or Cloning of Human Cells

MethoCult™ H4100 is recommended as a base medium for the preparation of methylcellulose-based medium. It is used for the culture of human cells 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 liquid culture media, cytokines, and other supplements to meet the specific requirements of investigators.

MethoCult™ H4100 contains only 2.6% methylcellulose in Iscove's MDM. A 1.0% concentration of methylcellulose is obtained when brought to a final volume of 100 mL.

Properties

Storage: Store at -20°C.
Shelf Life: Stable until expiry date (EXP) on label.
Contains:
- 2.6% Methylcellulose
- Iscove's MDM

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™ H4100 MEDIUM

MethoCult™ H4100 does not contain cytokines or other medium supplements. 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™ H4100 medium per bottle or per tube. The ratio (v:v) of MethoCult™ to other components in the liquid medium (e.g. cytokines) is important for viscosity, which ensures optimal CFU growth and morphology.

Use sterile techniques to prepare complete MethoCult™ H4100 medium (MethoCult™ H4100 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 40 mL bottle of MethoCult™ H4100 at room temperature (15 - 25°C) or overnight at 2 - 8°C.
   NOTE: Do not thaw MethoCult™ at 37°C.
2. Prepare desired growth factors, supplements, and Iscove's Modified Dulbecco's Medium (IMDM; Catalog #36150) in 60 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 40 mL bottle of MethoCult™ H4100 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™ H4100 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 (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™ H4100 (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™ H4100 base medium for duplicate or triplicate cultures (see Table 1 for required volumes), store at -20°C, then add desired components after thawing.

Table 1. Volumes Required for Preparation of Complete MethoCult™ H4100 Medium

COMPONENT	PER BOTTLE	PER TUBE (duplicate 1.1 mL cultures)	PER TUBE (triplicate 1.1 mL cultures)
MethoCult™ H4100	40 mL	1.2 mL	1.6 mL
IMDM with cytokines*	60 mL	1.8 mL	2.4 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 human CFU assays, and counting and classification of colonies, refer to the Technical Manual: Human Colony-Forming Cell Assays Using MethoCult™ (Document #28404), available at www.stemcell.com or contact us to request a copy.

References

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.