MethoCult™ H4100

Base Methylcellulose Medium for Human Cells

Catalog #04100 40 mL

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Product Description

Incomplete Methylcellulose-Based Medium for Colony-Forming Unit (CFU) Assays or Cloning of Human Cells

MethoCult™ H4100 is recommended as a base medium to detect and quantify hematopoietic progenitors 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 (IMDM). A 1% 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 in Iscove's MDM

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™ 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 aliquot methylcellulose as the volume dispensed will not be accurate. Use of blunt-end needles for dispensing prevents needle-stick injuries.

A. TO PREPARE 100 ML BOTTLE

1. Thaw 40 mL bottle 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 MDM (IMDM) with 25 mM HEPES (Catalog #36150) in a volume of 60 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 40 mL bottle 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 1.2 mL per tube for 1.1 mL duplicate cultures or 1.6 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 IMDM with 25mM HEPES (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 our website at www.stemcell.com.

For recommended cell plating concentrations, set-up 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

Eaves CJ, Eaves AC: Anatomy and physiology of hematopoiesis. In: Childhood Leukemias (2nd Edition) (CH Pui, ed.), Cambridge University Press, Cambridge, pp 69-105, 2006

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

Wognum B, Yuan N, Lai B, & Miller CL: Colony Forming Cell Assays for Human Hematopoietic Progenitor Cells. In: Basic Cell Culture Protocols (CD Helgason, CL Miller, eds.), Humana Press Inc., Clifton, New Jersey, p267-83, 2013.

Nissen-Druey C, Tichelli A, Meyer-Monard S: Human Hematopoietic Colonies in Health and Disease, S. Karger Medical and Scientific Publishers, Basel, 2005. Reprint of Acta Haematol 113: 5-96, 2005 (Catalog #28760)