

MethoCult™ H4230

Methylcellulose-based medium without cytokines for human cells

Catalog #04230

80 mL



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Product Description

Colony-Forming Unit (CFU) Assays for Human Cells

MethoCult™ H4230 is recommended as a base medium for the culture of human cells to detect and quantify hematopoietic progenitor cells in human bone marrow, mobilized peripheral blood, peripheral blood, and cord blood samples using CFU assays. This formulation allows for the addition of an exogenous source of erythropoietin (EPO) and other cytokines to meet the specific requirements of investigators.

MethoCult™ H4230 is also useful for other applications, including cloning cell lines.

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™ H4230 MEDIUM

MethoCult™ H4230 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™ H4230 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™ H4230 medium (MethoCult™ H4230 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™ H4230 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 culturesComplete MethoCult™ medium is now ready for use.

B. TO PREPARE INDIVIDUAL TUBES

1. Thaw MethoCult™ H4230 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™ H4230 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™ H4230 (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™ H4230 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™ H4230 Medium

COMPONENT	PER BOTTLE	PER TUBE (duplicate 1.1 ml cultures)	PER TUBE (triplicate 1.1 ml cultures)
MethoCult™ H4230	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 human CFU assays, and counting and classification of colonies, refer to the Technical Manual: Human Colony-Forming Unit (CFU) Assays Using MethoCult™ (Document # 10000005589), 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

- 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.

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