MethoCult™ GF H84534

Methylcellulose medium with recombinant cytokines

Catalog #84534 100 mL

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

MethoCult™ GF H84534 is used in colony-forming unit (CFU) assays to detect and quantify human hematopoietic progenitor cells in bone marrow (BM), mobilized peripheral blood (MPB), peripheral blood (PB), and cord blood (CB) samples. It is recommended for CD34+ enriched cells, mononuclear cells, and cells isolated by other purification methods.

MethoCult™ GF H84534 has been formulated to support optimal growth of granulocyte-macrophage progenitor cells (CFU-GM, CFU-M, and CFU G).

Quality Control

MethoCult™ methylcellulose-based media are manufactured using aseptic technique, tightly controlled processes, and extensively pre-screened components. Each batch of MethoCult™ is sterility tested according to USP methods and Quality Control performance tested in CFU assays using human BM, CB, or PB samples. A Certificate of Analysis is available upon request.

Properties

Storage: Store at -15 to -25°C.

NOTE: Product may be shipped with dry ice or ice packs and may be received thawed. If product is received partially thawed, place immediately at -20°C or thaw and aliquot as described in “Handling/Directions for Use”.

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 stem cell factor (SCF)
- Recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF)
- Recombinant human granulocyte colony-stimulating factor (G-CSF)
- Recombinant human interleukin 3 (IL-3)

Special Materials Required but Not Provided

EQUIPMENT

- Biohazard Safety Cabinet certified for Level II handling of biological materials. *All procedures for cell processing and setup of CFU assays should be performed using sterile technique and universal safe handling precautions.*
- Incubator set at 37°C with 5% CO_2 in air and ≥ 95% humidity. *Use of water-jacketed incubators with a water pan placed in the chamber is recommended.*
- Inverted Microscope. *Use of a high-quality inverted microscope equipped with a 10X or 12.5X eyepiece objective, 2X, 4X, and 10X planar objectives, and a blue filter is recommended.*
- The STEMvision™ instrument for automated imaging and counting of hematopoietic colonies may be used in place of a microscope to count colonies. See www.stemvision.com for more details.
- Equipment for cell processing and cell counting as required.

REAGENTS AND MATERIALS

- MethoCult™ Cell Wash Medium (Catalog #87700)
- 16 Gauge Blunt-End Needles (Catalog #28110)*
- 35 mm Culture Dishes (Catalog #27100)* or SmartDish™ 6-well culture plates (Catalog #27370)
- Corning® 60 mm Gridded Scoring Dish (Catalog #100-0085)* or STEMgrid™-6 counting grid (Catalog #27000)
- Syringes (luer lock): 3 mL (Catalog #28230) or 6 mL

- Sterile pipettes and sterile polystyrene tubes
- 100 mm culture dishes (e.g. Tissue Culture-Treated Dishes, Catalog #27125)
- 245 mm x 245 mm square culture dishes (e.g. 245 mm Square Dish, Tissue-Culture Treated, Catalog #100-0084) or 150 mm culture dishes
- Sterile distilled water
- Cell processing and cell counting reagents and materials as required

** Use of STEMCELL Technologies products with the indicated catalog numbers is recommended (see Notes).*

Handling/Directions for Use

A. PREPARE METHOCULT™ MEDIUM

1. Thaw MethoCult™ methylcellulose medium under refrigeration (2 - 8°C) overnight or at room temperature (15 - 25°C). Avoid repeated freeze-thaw cycles.
2. Once thawed, 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, aliquot 3 mL per tube for 1.1 mL duplicate cultures or 4 mL per tube for 1.1 mL triplicate cultures. Tubes can be used immediately or stored at -20°C for later use.

   Do not use a standard pipette to aliquot methylcellulose as the volume dispensed will not be accurate. Use blunt-end needles for dispensing to prevent needle-stick injuries.

B. PREPARE CELL SAMPLE

1. The human cell source and cell sample processing method used is dependent on individual laboratory requirements.
2. It is recommended that cell samples are washed and diluted in MethoCult™ Cell Wash Medium.
3. The following are examples of suitable cell processing techniques:
   a. **Mononuclear cell suspensions** or light density cells prepared by density separation using reagents such as Lymphoprep™ (Catalog #07801).
   b. **Mobilized peripheral blood collections** prepared using an apheresis machine.
   c. **Red blood cell (RBC)-depleted cell suspensions** prepared by lysis or sedimentation of RBCs.
   d. **CD34+ enriched cells** prepared by methods including immunomagnetic cell separation and fluorescence-activated cell sorting (FACS).
4. Count nucleated cells using a hemocytometer after diluting with 3% Acetic Acid with Methylene Blue (Catalog #07060) or using an automated cell counter. Methods to assay viable cells (e.g. Trypan Blue [Catalog #07050] dye exclusion) should be used for cell preparations where a decrease in cell viability may be expected (e.g. cryopreserved cells).

C. SETUP OF CFU ASSAYS

1. Thaw tubes at room temperature (15 - 25°C) or overnight at 2 - 8°C.
2. **Dilute cells:** Prepare a 10X concentrated cell suspension (see Table 1 and Notes) of cells in MethoCult™ Cell Wash Medium. For example, prepare a sample of 5 x 10^5 cells/mL in MethoCult™ Cell Wash Medium for a plating concentration of 5 x 10^4 cells per dish.
3. Add 0.3 mL of cells to 3 mL of MethoCult™ for duplicate cultures, or 0.4 mL of cells to 4 mL of MethoCult™ for triplicate cultures.

   This 1:10 v/v ratio of cells:medium gives the correct medium viscosity to ensure optimal CFU growth and morphology.
4. Vortex tube to mix contents thoroughly and then let stand for 2 - 5 minutes to allow bubbles to rise to the top before dispensing.
5. **Dispense:** Using a 3 mL syringe attached to a 16 gauge blunt-end needle, dispense 1.1 mL of the MethoCult™ mixture containing cells into 2 (or 3) 35 mm dishes. Gently tilt and rotate each dish to distribute methylcellulose evenly.
6. **Add 3 mL** of sterile water to an additional uncovered 35 mm dish. For duplicate assays, place all three dishes into a 100 mm culture dish. For triplicate assays, place 35 mm dishes in cultureware with a loose-fitting lid (e.g. 150 mm square dishes, 245 mm x 245 mm square culture dishes).

   Always provide water dishes to maintain humidity.
7. **Incubate** at 37°C, in 5% CO_2, with ≥ 95% humidity for 14 - 16 days. Proper culture conditions are critical for optimal CFU growth. Use of water-jacketed incubators with water pan in chamber and routine monitoring of temperature and CO_2 levels is recommended (see Notes).

D. COLONY IDENTIFICATION AND COUNTING

Counting and classification of human colonies is performed after 14 - 16 days in culture.

COLONY COUNTING OVERVIEW

Use a high-quality inverted microscope equipped with 2X, 4X, and 10X planar objectives and stage holder for a 60 mm dish. First, scan the dish on low power (2X objective, 20 - 25X magnification) to evaluate the relative distribution of colonies. Count CFU GM, CFU-G, and CFU-M on low power. Use high power (4X objective, 40 - 50X magnification) to confirm colony type as required.

Colony Descriptions

CFU-GM: Colony-forming unit-granulocyte, macrophage produces a colony containing > 40 granulocyte and macrophage cells.

CFU-G and **CFU-M:** Colonies contain > 40 granulocytes and macrophages, respectively.

Notes

- Syringes and large bore blunt-end needles should be used for accurate dispensing of viscous methylcellulose medium and to prevent needle-stick injuries.
- It is important to use Petri dishes that have been pre-screened for low cell adherence, because excessive cell adherence can inhibit CFU growth or interfere with colony recognition.
- It is important to routinely monitor incubator temperature and CO_2 and humidity levels to ensure proper culture conditions.
- Fresh or cryopreserved cell samples can be used.
- Suitable cell processing procedures must be established in each laboratory. For example, fresh cord blood samples depleted of RBCs by sedimentation using HetaSep™ (Catalog #07806) may contain residual RBCs, which can interfere with colony detection and identification.
- Sufficient cells should be added to yield approximately 25 to 120 colonies per 35 mm dish (1.1 mL culture). Each laboratory should establish appropriate plating concentrations by setting up test cultures at two to four different cell concentrations.
- To facilitate identification and counting of colonies, assays may be set up in SmartDish™ cultureware instead of 35 mm dishes. STEMvision™ may then be used for automated counting. Alternatively, STEMgrid™-6 may be used to assist with manual counting.
- For additional assistance on hematopoietic colony recognition and counting, refer to the references listed below and the Technical Manual: Human Colony-Forming Unit (CFU) Assays Using MethoCult™ (Document #10000005589), available at www.stemcell.com.

Table 1. Recommended Cell Plating Concentrations

CELL SOURCE	CELLS PER 35 MM DISH
BM, ammonium chloride-treated	5 x 10^4 (2 x 10^4 - 1 x 10^5)
BM, light density	2 x 10^4 (1 - 5 x 10^4)
CB, light density	1 x 10^4 (5 x 10^3 - 2 x 10^4)
CB, RBC-depleted	5 x 10^4 (2 - 6 x 10^4)
PB, light density	2 x 10^5 (1 - 2 x 10^5)
MPB, light density	2 x 10^4 (1 - 5 x 10^4)
Lin-depleted (CD34+ enriched BM, CB, MPB)	1000 (500 - 2 x 10^3)
CD34+ cells (BM, CB, MPB)	500 (500 - 2 x 10^3)

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. (1995) Assays of hematopoietic progenitor cells. In: Beutler E, Lichtman MA, Coller BS, & Kipps TJ (Eds.). Williams Hematology Fifth Edition (pp. L22–6). New York: McGraw-Hill Inc.

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.

Technical Assistance

For technical support, contact us by email at techsupport@stemcell.com, or call +1.604.877.0713, or the European toll-free number 00800 7836 2355. For more information, visit www.stemcell.com.

If you require a printed copy or a translated version of this document in a certain language, contact us at techsupport@stemcell.com.