MethoCult™ GF H84444

Methylcellulose Medium with Recombinant Cytokines

REF **84444** **24 x 3 mL**

INTENDED USE

MethoCult™ GF H84444 is intended for use 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.

PRODUCT DESCRIPTION

MethoCult™ GF H84444 has been formulated to support optimal growth of erythroid progenitor cells (CFU-E and BFU-E), granulocyte-macrophage progenitor cells (CFU-GM, CFU-M, CFU-G) and multi-potential granulocyte, erythroid, macrophage, megakaryocyte progenitor cells (CFU-GEMM).

Components include:

- Iscove's MDM
- Methylcellulose
- Fetal bovine serum
- Bovine serum albumin
- Recombinant human (rh) stem cell factor
- rh GM-CSF
- rh G-CSF
- rh Interleukin 3
- rh Erythropoietin

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.

STABILITY AND STORAGE

Store at -15 to -25°C. Product stable at -15 to -25°C until expiry date (EXP) on label.

Do not repeatedly freeze and thaw.

If product is received partially thawed, place immediately at -20°C.

WARNINGS AND PRECAUTIONS

1. For professional use only.
2. This product is for in vitro diagnostic use.
3. This product should be handled by trained personnel observing good laboratory practices.
4. This product contains material of animal origin and should be handled as a potential carrier and transmitter of disease. Handling of reagents and disposal of waste should observe all local, state, or national regulations.
5. This product is a potential irritant to eyes, respiratory system, and skin. This product may also be harmful if ingested. Avoid exposure through skin, eye contact, inhalation, and ingestion. May cause allergic reaction in sensitized individuals.

SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED

Equipment

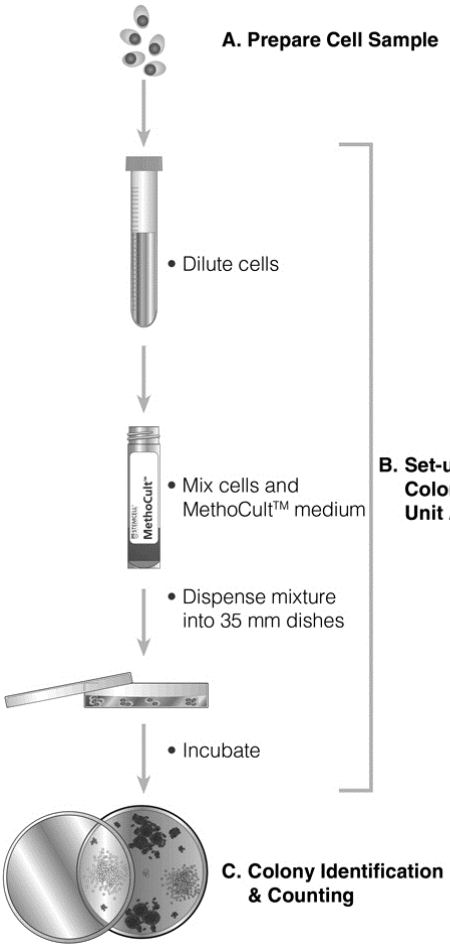
- Biohazard Safety Cabinet certified for Level II handling of biological materials. *All procedures for cell processing and setup of CFU assays should be performed using sterile technique and universal safe handling precautions.*
- Incubator set at 37°C with 5% CO_2 in air and ≥ 95% humidity. *Use of water-jacketed incubators with a water pan placed in the chamber is recommended.*
- Inverted Microscope. *Use of a 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.stemcell.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 #27301)
- 60 mm Gridded Scoring Dish (Catalog #27500)* or STEMgrid™-6 counting grid (Catalog #27000)
- Syringes (Luer lock): 3 mL (Catalog #28230), 6 mL
- Sterile pipettes and sterile polystyrene tubes
- 100 mm culture dishes (e.g., Treated Tissue Culture Dishes, Catalog #27125)
- 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 AND DIRECTIONS FOR USE



A. 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).

Lymphoprep is a trademark of AXIS-SHIELD.

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).*

B. Setup of Colony-Forming Unit Assays

1. Thaw tubes under refrigeration (2 - 8°C) overnight or at room temperature (15 - 25°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.

   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 two 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.

   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).*

C. 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 Gridded Scoring Dish. A blue filter will enhance the red color of hemoglobinized erythroblasts in CFU-E, BFU-E, and CFU-GEMM. First scan the dish on low power (2X objective, 20 - 25X magnification) to evaluate the relative distribution of colonies. Count CFU-E with 4X objective (40 - 50X magnification), and then BFU-E, CFU-GM, and CFU-GEMM on low or medium power. Use high power to confirm colony type as required.

COLONY DESCRIPTIONS

CFU-E: Colony-forming unit-erythroid produces a colony containing 1 to 2 clusters with a total of 8 - 200 erythroblasts.

BFU-E: Burst-forming unit-erythroid produces a colony containing > 200 erythroblasts, usually present in > 2 clusters.

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.

CFU-GEMM: Colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte produces a colony containing erythroid cells as well as 20 or more granulocyte, macrophage, and megakaryocyte cells.

NOTES

- Syringes and large bore blunt-end needles should be used for accurate dispensing of viscous methylcellulose medium and to prevent needle-stick injuries.
- 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.
- 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 #28404), available at www.stemcell.com.

For Technical Assistance

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Table 1. Recommended Cell Plating Concentrations

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

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 in your region, visit www.stemcell.com/primarycells.

REFERENCES

1. Eaves C. (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.
2. 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.
3. Eaves C & Lambie K. (1995) Atlas of Human Hematopoietic Colonies. Vancouver: STEMCELL Technologies Inc. *(Catalog #28700)*
4. Nissen-Druey C et al. (2005) Human hematopoietic colonies in health and disease. Basel, Switzerland: S. Karger Medical and Scientific Publishers. *(Catalog #28760)*

TECHNICAL ASSISTANCE

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

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REF Catalog or reference number	LOT Batch code	 Use by: YYYY-MM
 Caution, consult accompanying documents	IVD In Vitro Diagnostic Medical Device	 For storage within temperature limits
 Manufacturers identification (name & address)	EC REP Authorized EC representative in the European Community	CE CE Mark