MethoCult™ GF H84445

Methylcellulose Medium with Recombinant Cytokines

REF **84445** **24 x 3 mL**

INTENDED USE

MethoCult™ GF H84445 is intended for use in colony-forming unit (CFU) assays to detect and quantify human hematopoietic progenitors 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 H84445 has been formulated to support optimal growth of erythroid progenitors (CFU-E and BFU-E), granulocyte-macrophage progenitors (CFU-GM, CFU-M, CFU-G) and multi-potential granulocyte, erythroid, macrophage, megakaryocyte progenitors (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 Interleukin-6
- 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 or thaw and aliquot as described in “Handling and Directions for Use”.

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. Once human cells are combined with the product, treat as potentially biohazardous. Handling of reagents and disposal of waste should observe all local, state or national regulations.
4. 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

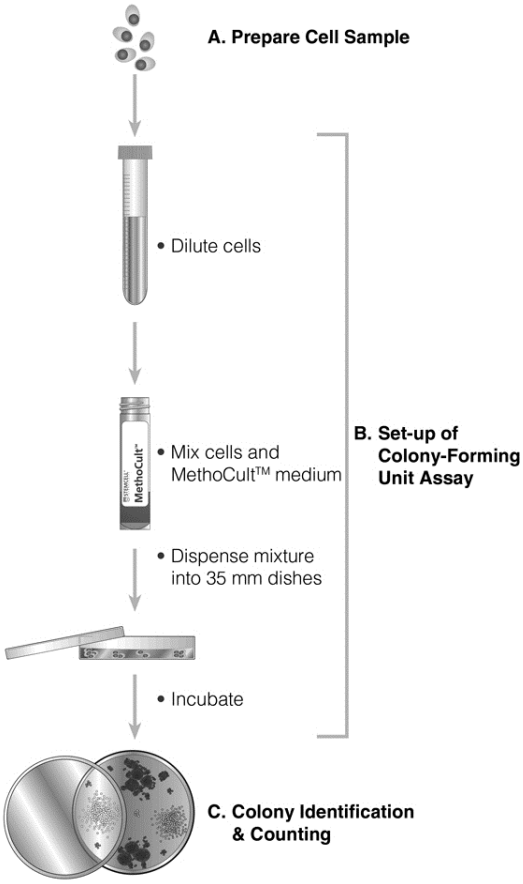
- Biohazard Safety Cabinet certified for Level II handling of biological materials. *All procedures for cell processing and set-up of CFU assays should be performed using sterile technique and universal safe handling precautions.*
- Incubator set at 37°C with 5% CO_2 in air and ≥95% humidity. *Use of water-jacket incubators with a water pan placed in the chamber is recommended.*
- Inverted Microscope. *Use of a quality inverted microscope equipped with a 10 or 12.5X eyepiece objective, 2X, 4X, and 10X planar objectives and a blue filter is recommended.*
- 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, 6 mL
- Sterile pipettes and sterile polystyrene tubes
- 100 mm culture dishes (e.g., Treated Tissue Culture Dishes, Catalog #27125)
- 245 mm x 245 mm square culture dishes (e.g., 245 mm x 245 mm Square Treated Tissue Culture Dishes, Catalog #27140) 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 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 Ficoll-Paque™.
   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 fluorescent activated cell sorting (FACS).

   Ficoll-Paque™ is a trademark of GE Healthcare Ltd.
4. Count nucleated cells using trypan blue dye exclusion, 3% acetic acid or automated cell counter. *Methods to assay viable cells (i.e. dye exclusion) should be used for cell*

preparations (i.e. cryopreserved cells, ex vivo manipulation) where a decrease in cell viability may be expected.

B. Set-up 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, 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 culture 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).*

C. Colony Identification and Counting

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

Scoring Overview

Use a high-quality inverted microscope equipped with 2X, 4X and 10X planar objectives and stage holder for a 60 mm gridded 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. Score CFU-E with 4X objective (40 - 50X magnification), and then BFU-E, CFU-GM and CFU-GEMM on low. 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, 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.
- For additional assistance on hematopoietic colony recognition and counting, refer to the references listed below and the Technical Manual: Human Colony - Forming Unit Assays Using MethoCult™ (Document #28404).

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 ($500 - 2 \times 10^3$)
$CD34^+$ cells (BM, CB, MPB)	500 ($500 - 2 \times 10^3$)

REFERENCES

1. Eaves CJ: Assays of hematopoietic progenitor cells. Williams Hematology, 5 (eds. E Beutler, MA Lichtman, BS Coller, TJ Kipps), McGraw-Hill, Inc., pp L22-6, 1995.
2. Wognum B, Yuan N, Lai B, Miller CL: Colony forming cell assays for human hematopoietic progenitor cells. Methods Mol Biol 946:267-283, 2013
3. Eaves C and Lambie K: Atlas of Human Hematopoietic Colonies. STEMCELL Technologies, Inc., 1995 *(Catalog #28700).*
4. Nissen-Druey C, Tichelli A and Meyer-Monard S: Human Hematopoietic Colonies in Health and Disease. S. Karger Medical and Scientific Publishers, 2005. Reprint of Acta Haematol 113 (1): 5-96, 2005 *(Catalog #28760).*

TECHNICAL ASSISTANCE

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

If you require a printed copy or a translated version of this document in a certain language please contact technical support.

EC REP **MDSS GmbH**

Schiffgraben 41

30175 Hannover, Germany

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 Mark