MethoCult™ Express

Methylcellulose Medium with Recombinant Cytokines

FOR RAPID HEMATOPOIETIC COLONY ASSAYS OF HUMAN CELLS

REF 04437	100 mL
REF 04447	24 x 3 mL

ENGLISH

INTENDED USE

MethoCult™ Express is intended for use in colony-forming unit (CFU) assays to detect and quantify human hematopoietic progenitors in cord blood (CB) samples after a minimum culture period of seven days. It is recommended for red blood cell (RBC)-depleted CB samples, whole CB samples that have been cryopreserved and thawed, and CB mononuclear cells.

PRODUCT DESCRIPTION

MethoCult™ Express is optimized for the detection and counting of human hematopoietic progenitor cells after much shorter periods than the 14 -16 days of standard CFU assays. In MethoCult™ Express, colonies containing at least 20 cells can be counted as early as after 7 days of culture. At this time, most colonies are immature and have not yet differentiated into morphologically distinguishable colony types. Therefore the colonies counted after seven days of culture give information about the total frequency of hematopoietic progenitor cells present in the sample without distinction between different progenitor types.

If MethoCult™ Express cultures are maintained for 14 - 16 days, colonies derived from erythroid progenitors (BFU-E); granulocyte/macrophage progenitors (CFU-GM, CFU-M, CFU-G); and multi-potential granulocyte, erythroid, macrophage and megakaryocyte progenitors (CFU-GEMM) can be counted.

Components include:

- Methylcellulose
- Fetal bovine serum
- Bovine serum albumin
- Cytokines, including erythropoietin (EPO)
- Supplements
- Iscove's MDM

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 CB 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.
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.

For Technical Assistance

Tel: +1.604.877.0713

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e-mail: techsupport@stemcell.com

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SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED

Equipment

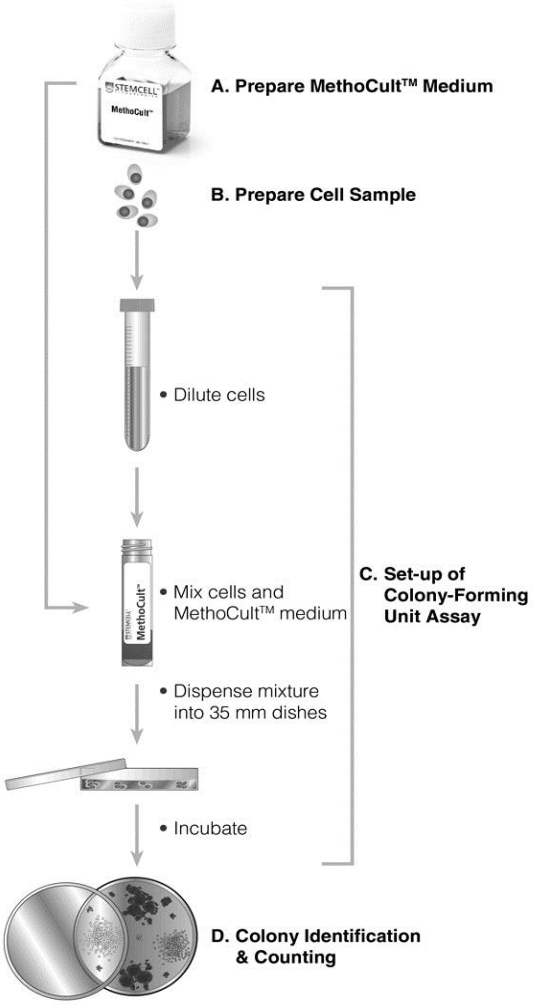
- Biohazard Safety Cabinet certified for Level II handling of biological materials. *All procedures for cell processing and set-up of CFU assays should be performed using sterile technique and universal safe handling precautions.*
- Incubator set at 37°C with 5% CO_2 in air and ≥ 95% humidity. *Use of 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.*
- The STEMvision™ instrument for automated imaging and scoring of hematopoietic colonies may be used in place of a microscope to score colonies in the 7-day CFU-assay. 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, 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



Catalog #04437 (100 mL): Steps A, B, C, & D
Catalog #04447 (24 x 3 mL): Steps B, C & D only

A. Prepare MethoCult™ Medium

1. Thaw MethoCult™ methylcellulose medium under refrigeration (2 - 8°C) overnight or at room temperature (15 - 25°C).
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.*

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. **Red blood cell-depleted cell suspensions** can be prepared by lysis of red blood cells (RBCs) using ammonium chloride solution (Catalog #07800), as described in the Technical Manual (Document #28404).
   b. **Whole cord blood** that has been **cryopreserved** can be **thawed** according to the procedure operational in your institution. A suggested procedure is provided in the Technical Manual (Document #28404). *Red blood cells and other mature cells (e.g. granulocytes) are sensitive to cryopreservation and will lyse, significantly reducing background in the dishes.*
   c. **Mononuclear cells** can be prepared by density centrifugation using a reagent such as Ficoll-Paque™.

   Ficoll-Paque™ is a trademark of GE Healthcare Ltd**.**
4. Count nucleated cells using a hemacytometer 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. 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) 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.

   The progenitor content and quality of individual cord blood preparations can be highly variable. Plate cells at 2 - 4 different densities to ensure sufficient cells are plated to yield approximately 25 - 120 colonies per 35 mm dish (1.1 mL culture).
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 (Catalog #27125). 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 7 days (or 14 - 16 days, if desired). 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

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 when counting colonies at 14 - 16 days.

Scoring After 7 Days

Scan the dish on low power (2X objective, 20 - 25X magnification) to evaluate the relative distribution of colonies. Score colonies with 4X objective and count all colonies containing more than 20 cells. As most colonies are immature, scoring individual colony types (i.e. BFU-E and CFU-GM) is not recommended after only 7 days. Please refer to the Technical Manual (Document #28404) for examples of colonies counted after 7 days of culture in MethoCult™ Express.

Scoring After 14 - 16 Days

NOTE: Cord blood-derived colonies in MethoCult™ Express can be very large after 14 days of culture and it may be difficult to accurately distinguish individual colonies in dishes plated at high cell concentrations. Plating at different cell concentrations is recommended to assess progenitor frequencies (see Table 1).

Mature BFU-E, CFU-GM and CFU-GEMM can be distinguished and counted using standard criteria. Refer to the Technical Manual Human Colony-Forming Cell Assays Using MethoCult™ (Document #28404), available at www.stemcell.com.

First, scan the dish on low power (2X objective, 20 - 25X magnification) to evaluate the relative distribution of colonies. Score BFU-E, CFU-GM and CFU-GEMM on low or medium power and use high power to confirm colony type as required.

COLONY DESCRIPTIONS

Scoring After 14 - 16 Days

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.

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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 RBC by sedimentation using HetaSep™ (Catalog #07806) may contain residual RBC, which can interfere with colony detection and identification.
- To facilitate identification and scoring of colonies, assays may be set up in SmartDish™ cultureware instead of 35 mm dishes. For automated counting, STEMvision™ may then be used with the MethoCult™ Express algorithm (for 7-day CFU assays). 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 Assays Using MethoCult™ (Document #28404).

  Available in English only.

Table 1. Recommended Cell Plating Concentrations

CELL SOURCE	CELLS PER 35 mm DISH
CB, RBC-depleted	2×10^4 - 5×10^4
Whole CB, cryopreserved	3×10^4 - 5×10^4
CB mononuclear cells	1×10^4 - 2×10^4

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. *Available in English only (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. *Available in English only (Catalog #28760).*
5. Atlas of Hematopoietic Colonies from Cord Blood. STEMCELL Technologies, www.stemcell.com. *Available in English only (Catalog #29940).*

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 please 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 Mark