

## MethoCult™ Express

### Methylcellulose Medium with Recombinant Cytokines

FOR RAPID HEMATOPOIETIC COLONY ASSAYS OF HUMAN CELLS

REF 04447

24 x 3 mL

#### ENGLISH

### INTENDED USE

MethoCult™ Express is intended for use in colony-forming cell (CFC) 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.

For professional *in vitro* diagnostic use only.

### 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 CFC 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

This product is a biological reagent, and as such cannot be completely characterized or quantified.

### QUALITY CONTROL

MethoCult™ methylcellulose-based media are aseptically manufactured using tightly controlled processes and extensively pre-screened components.

Each batch of MethoCult™ is sterility tested according to USP standards and Quality Control performance tested in CFC assays using human CB samples. A Certificate of Analysis is available upon request.

### LIMITATIONS

Product has been CE Marked in the European Union according to the 98/79/EC *In Vitro* Diagnostic Device Directive and complies with its requirements.

Additional terms and conditions may apply.

### STABILITY AND STORAGE

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

Do not repeatedly freeze and thaw.

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

### PRECAUTIONS

1. For professional use only.
2. 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.
3. 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.

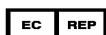


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## SPECIAL MATERIALS REQUIRED BUT NOT PROVIDED

### Equipment

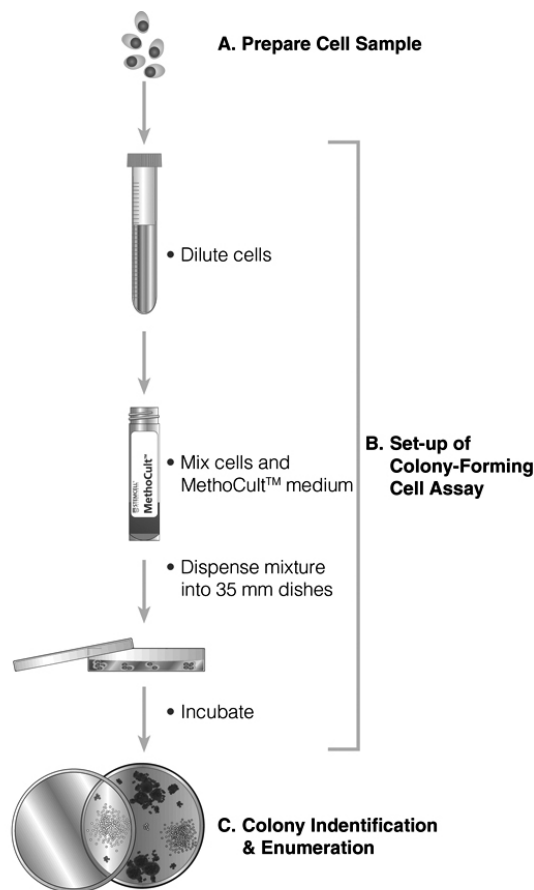
- Biohazard Safety Cabinet certified for Level II handling of biological materials. *All procedures for cell processing and set-up of CFC assays should be performed using sterile technique and universal safe handling precautions.*
- Incubator set at 37°C with 5% CO<sub>2</sub> in air and ≥95% humidity. *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 CFC-assay. See [www.stemcell.com](http://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 #28110/28120)\*
- 35 mm pre-tested Culture Dishes (Catalog #27100/27150)\*
- 60 mm Gridded Scoring Dish (Catalog #27500)\*
- Syringes (Luer lock): 3 mL, 6 mL
- Sterile pipettes and sterile polystyrene tubes
- 100 mm disposable petri 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



For more detailed instructions refer to the Technical Manual "Human Colony - Forming Cell Assays Using MethoCult™" (Manual Catalog #28404), available at [www.stemcell.com](http://www.stemcell.com).

Available in English only.

### 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 (Catalog #87700).
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 (RBC) using ammonium chloride solution (Catalog #07800/07850), as described in the Technical Manual (Manual Catalog #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 (Manual Catalog #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 centrifugation over Ficoll-Paque™ PLUS or PREMIUM.

Ficoll-Paque™ is a trademark of GE Healthcare Ltd.



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- 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 Cell Assays

- Thaw tubes under refrigeration (2 - 8°C) overnight or at room temperature (15 - 25°C).
- Dilute cells:** Prepare a 10X concentrated cell suspension (see Table 1) of cells in MethoCult™ Cell Wash Medium (Catalog #87700). For example, prepare a sample of  $5 \times 10^5$  cells/mL in MethoCult™ Cell Wash Medium for a plating concentration of  $5 \times 10^4$  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).*

- Add 0.3 mL of cells to 3 mL of MethoCult™.  
*This 1:10 v/v ratio of cells:medium gives the correct medium viscosity to ensure optimal CFC growth and morphology.*
- Vortex tube to mix contents thoroughly and then let stand for 2 - 5 minutes to allow bubbles to rise to the top before dispensing.
- 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 (Catalog #27100/27150). Gently tilt and rotate each dish to distribute methylcellulose evenly.
- 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 #27125/27127).  
*Always provide water dishes to maintain humidity.*
- Incubate** at 37°C, in 5% CO<sub>2</sub>, with ≥95% humidity for 7 days (or 14 - 16 days, if desired). Proper culture conditions are critical for optimal CFC growth. *Use of water-jacketed incubators with water pan in chamber and routine monitoring of temperature and CO<sub>2</sub> levels is recommended (see Notes).*

## C. Colony Identification and Enumeration

### 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 (Catalog #27500). 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 (Manual Catalog #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™" (Manual Catalog #28404), available at [www.stemcell.com](http://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

1. Syringes and large bore blunt-end needles should be used for accurate dispensing of viscous methylcellulose medium and to prevent needle-stick injuries.
2. Important to use petri dishes that have been pre-screened for low cell adherence because excessive cell adherence can inhibit CFC growth or interfere with colony recognition.
3. Important to routinely monitor incubator temperature, CO<sub>2</sub> and humidity levels to ensure proper culture conditions.
4. Fresh or cryopreserved cell samples can be used.
5. Suitable cell processing procedures must be established in each laboratory. For example, fresh cord blood samples depleted of RBC by sedimentation using HetaSep™ (Catalog #07806/07906) may contain residual RBC, which can interfere with colony detection and identification.
6. Automated identification and scoring of colonies after 7 days of culture can be performed using STEMvision™ with MethoCult™ Express algorithm (for 7-day CFC-assays). For use with STEMvision™, assays need to be set up in SmartDish™ cultureware instead of 35 mm dishes. Visit [www.stemcell.com](http://www.stemcell.com) for more details.
7. For additional assistance on hematopoietic colony recognition and enumeration, refer to the references listed below and the Technical Manual "Human Colony - Forming Cell Assays Using MethoCult™" (Manual Catalog #28404).

*Available in English only.*

**Table 1. Recommended Cell Plating Concentrations**

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

## 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. Eaves C and Lambie K: Atlas of Human Hematopoietic Colonies. STEMCELL Technologies, Inc., 1995. *Available in English only (Catalog #28700).*
3. 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).*
4. Atlas of Hematopoietic Colonies from Cord Blood. STEMCELL Technologies, Catalog # 29940, [www.stemcell.com](http://www.stemcell.com). *Available in English only.*






## TECHNICAL ASSISTANCE

For technical support please contact us by email at [info@stemcell.com](mailto:info@stemcell.com) or call **+1.604.877.0713**. For more information please visit [www.stemcell.com](http://www.stemcell.com).

**EC REP MDSS GmbH**

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

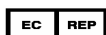


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