

RECOMMENDED FOR

Colony-Forming Cell (CFC) Assays for Human Cells

MethoCult[®] Express is recommended for the detection and counting of human hematopoietic progenitors in cord blood (CB) samples using CFC assays 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.

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 seven 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.

PRODUCT DESCRIPTION

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.

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

Each batch of MethoCult[®] is sterility tested and Quality Control performance tested in CFC assays using human CB samples. A Certificate of Analysis is available upon request.

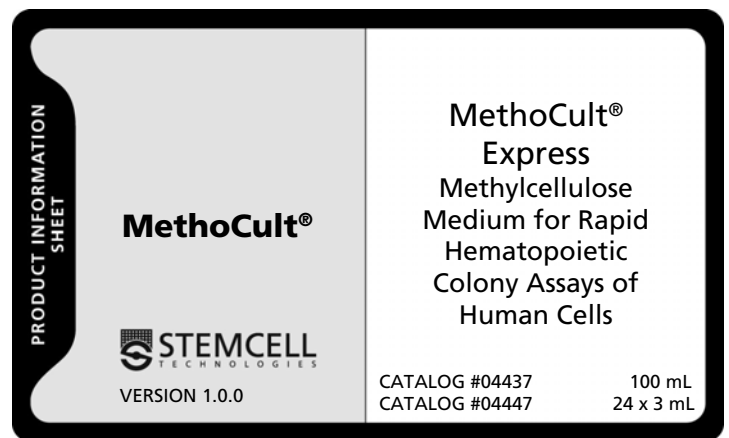
STABILITY AND STORAGE

Store at -20°C (-25°C to -15°C).

Product stable at -20°C until expiry date indicated on label. Stable for one month if stored at 2 - 8°C.

Avoid freezing and thawing repeatedly.

If product is received partially thawed, place immediately at -20°C or thaw and aliquot as described in 'Handling and Directions for Use'.



HANDLING AND DIRECTIONS FOR USE

For more detailed instructions refer to the Technical Manual for Human Colony-Forming Cell Assays Using MethoCult[®] Express (Manual Catalog #29926), available upon request and on our website at www.stemcell.com/technical/manuals.aspx.

Thawing and Dispensing Bottles of MethoCult[®]

1. Thaw MethoCult[®] 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 (Catalog #28110/28120), 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.*

Set-up of Human Colony-Forming Cell Assays

1. Thaw tubes under refrigeration (2 - 8°C) overnight or at room temperature (15 - 25°C).
2. Isolate cells according to the procedure operational in your institution or as described in detail in the Technical Manual (Manual Catalog #29926).

Red blood cell depleted cell suspensions can be prepared by lysis of red blood cells (RBC) using ammonium chloride solution (Catalog #07800/07806), as described in the Technical Manual (Manual Catalog #29926).

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 #29926). *Red blood cells and other mature cells (e.g. granulocytes) are sensitive to cryopreservation and will lyse, significantly reducing background in the dishes.*

3. Count nucleated cells using trypan blue (Catalog #07050) dye exclusion, 3% acetic acid with methylene blue (Catalog #07060) or automated cell counter. *Methods to assay viable cells (e.g. dye exclusion) should be used for cell preparations where a decrease in cell viability may be expected (e.g. cryopreserved cells).*



4. Prepare a 10X concentrated cell suspension (see Table 1) of cells in Iscove's MDM with 2% FBS (Catalog #07700). *For example, prepare a sample of 5×10^5 cells/mL in IMDM with 2% FBS for a plating concentration of 5×10^4 cells per dish.*
5. 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 viscosity to ensure optimal CFC growth and morphology.*
6. Vortex tube to mix contents thoroughly and then let stand for 2 - 5 minutes to allow bubbles to rise to the top before dispensing.
7. 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. *Dishes are pre-screened to ensure low cell adherence. Cell adherence can inhibit CFC growth.*
8. Add 3 - 4 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). For triplicate assays, place 35 mm dishes in cultureware with a loose-fitting lid (e.g. 150 mm dishes, square bacterial dishes). *Always provide water dishes to maintain humidity.*
9. Incubate at 37°C, in 5% CO₂, 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 CO₂ levels is recommended.

COUNTING AND CLASSIFICATION OF HUMAN COLONIES

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 7 days. Please refer to the Technical Manual (Manual Catalog #29926) 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 for Human Colony-Forming Cell Assays Using MethoCult[®] (Manual Catalog #28404), available upon request and on our website at www.stemcell.com/technical/manuals.aspx.

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 for 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.

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 |

The progenitor content and quality of individual cord blood preparations can be highly variable. Plate cells at 2 different densities to ensure sufficient cells are plated to yield 25 - 75 colonies per 35 mm dish (1.1 mL culture).

*Mononuclear cells (MNCs) are isolated by density-based cell separation (e.g. sedimentation over Ficoll-Paque™ PLUS). Ficoll-Paque™ PLUS is a trademark of GE Healthcare Ltd.