MethoCult™ GF R3774

Methylcellulose-based medium with recombinant cytokines (without erythropoietin [EPO]) for rat cells

Catalog # 03774 100 mL

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Product Description

Complete Methylcellulose-Based Medium for Colony-Forming Unit (CFU) Assays for Rat Cells
MethoCult™ GF R3774 is recommended for the detection and quantification of rat colony-forming unit granulocyte-macrophage progenitor cells (CFU-GM, CFU-G, CFU-M) in bone marrow (BM) samples.

Properties

Storage: Store at -20°C.

Shelf Life: Stable until expiry date (EXP) on label.

Contains:
- Methylcellulose in Iscove's MDM
- Fetal bovine serum
- Bovine serum albumin
- 2-Mercaptoethanol
- Stem cell factor (SCF)
- Interleukin 3 (IL-3)
- Granulocyte-macrophage colony-stimulating factor (GM-CSF)
- Supplements

Handling / Directions For Use

NOTE: If product is received partially thawed, place immediately at -20°C or thaw and aliquot as described below. Do not use MethoCult™ past the expiry date as indicated on the label.

NOTE: Do not use pipettes to dispense methylcellulose as the volume dispensed will not be accurate. Syringes and large bore blunt-end needles should be used for accurate dispensing of viscous methylcellulose medium and to prevent needle-stick injuries.

THAWING AND DISPENSING BOTTLES

1. Thaw 100 mL bottle of MethoCult™ GF R3774 at room temperature (15 - 25°C) or overnight at 2 - 8°C.
   NOTE: Do not thaw MethoCult™ at 37°C.
2. Shake vigorously for 1 - 2 minutes and then let stand for at least 5 minutes to allow bubbles to dissipate before aliquoting.
3. Using a 3 or 6 mL luer lock syringe attached to a 16 Gauge Blunt-End Needle (Catalog #28110), 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, stored at 2 - 8°C for up to 1 month, or stored at -20°C. After thawing aliquoted tubes of MethoCult™, mix well and use immediately. Do not re-freeze.

For further information, please refer to the Technical Bulletin: MethoCult™ for Rat Hematopoietic Colony-Forming Unit Assays (Document #28732), available at www.stemcell.com or contact us to request a copy.

COLLECTION OF RAT BM CELLS

NOTE: Suitable media for isolating cells include Iscove's Modified Dulbecco's Medium (IMDM; Catalog #36150), Iscove's MDM with 2% FBS (Catalog #07700), Alpha MEM Without Nucleosides (Catalog #36453), Dulbecco's Phosphate Buffered Saline (D-PBS) with 2% Fetal Bovine Serum (Catalog #07905), or D-PBS (Without Ca++ and Mg++; Catalog #37350).
Isolate cells in small volumes of medium.

1. Sacrifice rat according to protocols approved by your institution.
2. Wet fur thoroughly with 70% isopropyl alcohol.
3. Cut slit in pelt, taking care not to cut peritoneal membrane and peel back pelt to fully expose hind limbs.

4. Using sterile sharp dissecting scissors, cut at knee joint, and the femur near hip joint. Free tibia by cutting near ankle joint. Remove excess tissue and place bones in sterile culture dish or tube.
5. Trim ends of bones with sharp scissors to expose marrow shaft. Using a 3 mL syringe and 18 gauge needle with 2 - 3 mL of cold medium, insert needle into marrow shaft at knee joint end of femur and flush marrow into a sterile tube. Repeat flushing as required.
6. Prepare a single-cell suspension by gently drawing medium/cell suspension up and down several times using the syringe/needle.

SETUP OF RAT CFU-GM ASSAY

1. Thaw tubes of MethoCult™ at room temperature (15 - 25°C) or overnight at 2 - 8°C.
   NOTE: Do not thaw MethoCult™ at 37°C.
2. Prepare rat BM cell suspension and count nucleated cells using Trypan Blue (Catalog #07050) dye exclusion, 3% Acetic Acid with Methylene Blue (Catalog #07060), or an automated cell counter.
3. Prepare a 10X concentrated cell suspension in Iscove's MDM with 2% FBS.
4. Add 0.3 mL of cells to 3 mL of MethoCult™ (duplicate culture) or 0.4 mL to 4 mL of MethoCult™ (triplicate culture) to give a final density of 1.5 x 10^4 cells per 1.1 mL culture. This 1:10 (v/v) ratio of cells:medium gives the correct viscosity to ensure optimal CFU growth and morphology.
5. Vortex tube to mix thoroughly and then let stand for at least 5 minutes to allow bubbles to rise to the top before dispensing.
6. Dispense 1.1 mL of the MethoCult™ mixture containing cells using a 3 mL syringe attached to a 16 Gauge Blunt-End Needle to each of 2 or 3x 35 mm Culture Dishes (Catalog #27100). Dishes are pre-screened to ensure low cell adherence, which can inhibit CFU growth. Gently tilt and rotate each dish to distribute methylcellulose evenly.
7. Add 3 - 4 mL of sterile water to an uncovered 35 mm dish. For duplicate assays, place all 3 dishes into a 100 mm Treated Tissue Culture Dish (Catalog #27125). 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 for humidity.
8. Incubate at 37°C, 5% CO_2, with ~95% humidity for 9 - 14 days.
   NOTE: 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.

CELL PLATING CONCENTRATIONS

- Recommended plating concentration for fresh rat bone marrow cells is 1.5 x 10^4 per 1.1 mL culture in 35 mm dishes.
- Suitable plating concentrations for cryopreserved cells, cultured cells, and other hematopoietic tissues can be established by setting up cultures at 2 - 4 different cell concentrations
- The expected number of CFU-GM in fresh rat bone marrow samples is 407 +/- 63 per 1 x 10^5 BM cells.

COUNTING AND CLASSIFICATION OF RAT COLONIES

Colony Counting Overview:

Use a high-quality inverted microscope equipped with 2X, 4X, and 10X planar objectives and stage holder for a 60 mm dish. First scan the dish on low power (2X objective, 20 - 25X magnification) to evaluate the relative distribution of colonies. Count CFU-GM, CFU-G, and CFU-M on low power. Use high power to confirm colony type as required.

Colony Descriptions:

CFU-GM: Produces a colony containing > 30 granulocyte and macrophage cells.

CFU-G and CFU-M: Colonies contain > 30 granulocytes and macrophages, respectively.