ProductInformation

STEMLINE™ METHYLCELLULOSE MEDIUM, REDUCED GROWTH FACTOR MODIFICATION, HUMAN
Product Code **S 4187**
Storage Temperature -20 °C

Product Description
Semi-solid media supplemented with cytokines, maximize growth and differentiation of progenitor cells of interest and allow the clonal progeny of a single cell to stay together in a distinct cluster or colony.[1-5] These methylcellulose-based culture media have become the standard media used for the enumeration and evaluation of stem cell-derived progeny characterized as Colony Forming Units (CFU). Though such systems have been in existence for some time, problems with variations in media formulations have resulted in inconsistent product performance.

Stemline™ Methylcellulose Medium, Reduced Growth Factor Modification is a standardized, aseptically processed, semi-solid medium that provides reliable and predictable results for the culture of human hematopoietic cells. The medium is produced using a tightly controlled manufacturing process and rigorously-qualified raw materials to provide a consistent and optimally performing CFU assay system. This product is recommended for assay of human clonogenic hematopoietic progenitor cells from bone marrow, peripheral blood, leukopheresis products and CD 34+. This formulation will support the growth of granulocyte colonies (CFU-G), macrophage colonies (CFU-M), granulocyte/macrophage colonies (CFU-GM), erythroid colonies (BFU-E and CFU-E), and pluripotent mixed colonies (CFU-GEMM) [6]. All batches are performance tested in a 12- to 16 -day CFU assay using normal human CD 34+ progenitor cells.

Intended Use
For research use only. Not for diagnostic use in humans or animals.

Components
The medium consists of Iscove's Modified Dulbecco's Medium (IMDM), Pharmaceutical Grade Methylcellulose (1%; 4000 cps), Fetal Bovine Serum (FBS; 30%), Bovine Serum Albumin (1%), 2 mM L-Glutamine, 2-Mercaptoethanol (10^{-4} M), Stem Cell Factor (50 ng/ml), GM-CSF (10 ng/ml), IL-3 (10 ng/ml), and Erythropoietin (3 U/ml).

Storage/Stability
This product can be stored at –20 °C for up to two years after the date of manufacture. Expiration date appears on the product label. It is recommended that upon receipt, the Methylcellulose Medium be thawed overnight at 4 °C, aliquoted, and frozen at- 20 °C until time of use.

Procedure
Procedure for the Isolation of Mononuclear Cells (modification of method described by Fauser and Messner [2])

1. Collect fresh bone marrow, cord blood or peripheral blood into a tube containing an anticoagulant (EDTA, heparin, ACD) or use de-fibrinated blood.
2. Dilute the sample by addition of an equal volume of HBSS without Ca^{++} and Mg^{++} (Product Code: H 9394).
3. In a 50 ml sterile, conical polypropylene tube, slowly layer up to 20 ml of diluted sample onto 15 ml of a sterile density gradient separation medium of 1.077 g/ml (Histopaque-1077, Product Code: H 8889). The diluted sample and separation medium must be at room temperature to ensure optimal yield of mononuclear cells (MNCs) and efficient elimination of red blood cells.
4. Centrifuge the tube(s) at 400 x g for 30 minutes at room temperature.
5. Using a sterile Pasteur pipette, harvest the mononuclear cells from the sample/separation medium interface and transfer to a sterile 15 ml sterile, conical polypropylene tube. This MNC layer will contain monocytes, lymphocytes, platelets and hematopoietic progenitors.
6. Wash and then re-suspend the MNC fraction.
7. Adjust to the appropriate concentration, depending on sample source, with dilution medium (20% FBS/IMDM) as outlined in the Table below.

***NOTE**: It is important to remember that the sample must be adjusted to a concentration, which is 10-fold higher than the "Recommended Final Plating Concentration".*

Sample Type	**Recommended Final Plating Concentration** (per 1.1 ml)
Normal Bone Marrow (NH_4Cl-treated)	1×10^5 cells
Bone Marrow (Light Density)	1×10^4 cells
Normal Marrow $CD34^+$	1×10^3 cells
Cord blood (Light Density)	$5 \times 10^3 – 2 \times 10^5$ cells
Peripheral blood (Light Density)	2×10^5 cells
Mobilized Peripheral Blood	$5 \times 10^3 – 2 \times 10^5$ cells

8. For each sample, combine the following in a 15-ml sterile, polypropylene tube immediately prior to use.

a. 0.3 ml cells in dilution medium (20% FBS/IMDM).
b. 3.0 ml of Stemline™ Methylcellulose Medium, Reduced Growth Factor Modification, Human

9. The resulting 3.3-ml volume provides a triplicate of 35-mm plates to be set up, each containing 1.1 ml.
10. Vortex the tube vigorously until the cells are uniformly suspended.
11. After vortexing, let the tubes set for ~ 5 minutes to allow air bubbles to rise.
12. Using a sterile, 3-ml syringe fitted with a 16-gauge blunt tipped needle, aliquot 1.0 ml of the cell/methylcellulose suspension into each of three sterile 35-mm dishes. Place pairs of 35-mm dishes in a 100-mm dish. Add a third 35-mm dish (without a lid) containing sterile water to the plate in order to maintain an adequately humidified atmosphere.
13. Incubate the plate for 12 - 16 days in a humidified incubator at 37 ^{o}C and 5-10% CO_2. It is advisable to place the plate in an area that will not be disturbed for the duration of incubation.

Reading the plate:

The various hematopoietic colonies can be recognized by their color and morphology using an inverted microscope or a stereoscope. A colony consists of at least 40 cells. [2]

- CFU-GM is typically a flat colony consisting of translucent cells.
- BFU-E generally has densely packed sub-clusters of hemoglobin-containing cells ranging from pink to dark red/orange.
- CFU-GEMM has a “fried egg” appearance with a compact area that is usually central to (but may be to the side of) a peripheral flat lawn of translucent cells that may be either large or small.

References

1. Botnick, L.E., et al., Nature of the hemopoietic stem cell compartment and its proliferative potential. Blood Cells, **5,**195-210 (1979).
2. Dexter, T.M., Stromal cell associated haemopoiesis. J. Cell Physiol. Suppl., **1,** 87-94 (1982).
3. Dexter T.M., et al., Conditions controlling the proliferation of haemopoietic stem cells in vitro. J. Cell Physiol., **91,** 335-344 (1977).
4. Gordon, M.Y., et al., Compartmentalization of a haematopoietic growth factor (GM-CSF) by glycosaminoglycans in the bone marrow microenvironment. Nature, **326**, 403-405 (1987).
5. Dexter, T.M., Growth factors involved in haemopoiesis. J. Cell Sci., **88**, 1-6 (1987).
6. Williams, D.A. in *Hematology: Basic Principles and Practice*, Hoffman et al., (Eds.), pp. 126-138 (Churchill Livingstone, 2000).

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