Agar Leukocyte Conditioned Medium

Source of colony-stimulating factors for assays of human hematopoietic progenitor cells

Catalog # 02300 25 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

Agar Leukocyte Conditioned Medium (Agar-LCM) is a source of colony-stimulating factors for assays of human hematopoietic progenitor cells from light density fractions of bone marrow and peripheral blood. It is suitable for the growth of colony-forming unit erythroid (CFU-E), burst-forming unit-erythroid (BFU-E), colony-forming unit-macrophage (CFU-M), colony-forming unit-granulocyte (CFU-G), colony-forming unit-granulocyte, macrophage (CFU-GM) and colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte (CFU-GEMM) when added to MethoCult™ medium at a final concentration of 10% with the addition of erythropoietin (EPO; Catalog #02625). Agar-LCM is prepared using normal human peripheral blood leukocytes in an agar-containing medium.

Properties

Storage: Store at -20°C.

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

Contains:
- Agar Leukocyte Conditioned Medium
- Fetal bovine serum (10%)
- Iscove's MDM
- 1 x 10^4 2-Mercaptoethanol (ME)

Please refer to the Safety Data Sheet (SDS) for hazard information.

Handling / Directions For Use

Thaw Agar-LCM at room temperature (15 - 25°C) or overnight at 2 - 8°C.
NOTE: After thawing, some particulate matter may appear; the product may be clarified by centrifugation. There should be no loss in bioactivity as a result of this procedure. Keep on ice while in use.

Preparation of MethoCult™ Methylcellulose-based Medium Containing Agar-LCM for Detection of Human Hematopoietic Progenitor Cells (BFU-E, CFU-GM, CFU-GEMM):

1. Thaw 80 mL bottle of MethoCult™ H4230 (Catalog #04230) at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix contents by vigorous shaking.
2. Add 10 mL of Agar-LCM. Add EPO to yield a final concentration of 3 U/mL. Add Iscove's Modified Dulbecco's Medium (IMDM; Catalog #36150) to give a total volume of 100 mL.
3. Mix medium by vigorous shaking for 1 - 2 minutes. Let stand for 5 minutes to allow bubbles to rise to the top.
4. Dispense 3 mL of medium into sterile culture tubes using a 6 or 12 mL syringe attached to a 16 Gauge Blunt-End Needle (Catalog #28110).
   NOTE: If not used immediately, cap tubes tightly and store at -20°C. Thaw tubes at room temperature (15 - 25°C).
5. Prepare a suspension of human cells in Iscove's MDM with 2% FBS (Catalog #07700) at 10X the final concentration required for plating.
6. Add 0.3 mL of cells to 3.0 mL of MethoCult™ medium.
   NOTE: The addition of cells at a 1:10 (v/v) ratio to methylcellulose-based medium maintains optimal medium viscosity.
7. Vortex tubes to ensure contents are thoroughly mixed. Let stand for 2 - 3 minutes to allow bubbles to rise to the top.
8. Dispense 1.1 mL of MethoCult™ mixture containing cells into each of two 35 mm Culture Dishes (Catalog #27100) using a 3 mL syringe attached to a 16 Gauge Blunt-End Needle.
9. Incubate for 12 - 14 days at 37°C in 5% CO_2 with ≥ 95% humidity.
10. Score colonies using an inverted microscope.

NOTE: For additional information refer to the Technical Manual: Human Colony-Forming Unit (CFU) Assays Using MethoCult™ (Document #28404), available on our website at www.stemcell.com or contact us to request a copy.