MegaCult™-C Medium with Lipids

For assays of human or mouse megakaryocyte progenitor cells

Catalog #04850	50 mL
#04974	1 Kit
#04902	35 mL

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Product Description

MegaCult™-C Medium with Lipids is optimized for the detection and quantification of human megakaryocyte progenitor cells in bone marrow, mobilized peripheral blood, and cord blood. It is suitable for mononuclear cells, and recommended for CD34+ enriched cells and cells isolated by other purification methods. It can also be used for assays of megakaryocyte progenitor cells in unseparated or purified cell suspensions of mouse bone marrow.

MegaCult™-C Medium with Lipids (Catalog #04850) is formulated to support optimal growth of megakaryocytic progenitor cells (CFU-Mk). Addition of cytokines is required.

MegaCult™-C Collagen and Medium with Lipids kit (Catalog #04974) includes Collagen Solution (Catalog #04902) and MegaCult™-C Medium with Lipids. Addition of cytokines is required.

Properties

Storage: Store at -20°C.

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

Contains:

- Iscove's MDM
- Bovine serum albumin
- Recombinant human (rh) insulin
- Human transferrin (iron-saturated)
- Lipid solution
- 2-Mercaptoethanol
- L-Glutamine

This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Directions for Use

For complete instructions, refer to the Technical Manual: MegaCult™-C Assays for Quantitation of Human and Mouse Megakaryocytic Progenitor Cells (Document #10000005592), available at www.stemcell.com, or contact us to request a copy.

A. PREPARATION OF 50 mL BOTTLE

1. Thaw bottle of MegaCult™-C Medium with Lipids at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix well.
2. Aliquot the desired volume into tubes (e.g. 1.7 mL/tube).
3. Use immediately (section B step 2) or store at -20°C and thaw when required (section B step 1).

B. CULTURE PROCEDURE

1. Thaw tubes of MegaCult™-C Medium with Lipids at room temperature (15 - 25°C) or overnight at 2 - 8°C. Place thawed medium and Collagen Solution on ice.

   NOTE: If not used immediately, store tubes of MegaCult™-C Medium with Lipids at 2 - 8°C for up to 2 weeks.
2. Prepare a mixture of recombinant cytokines at 11X the desired final concentration in Iscove's Modified Dulbecco's Medium (Iscove's MDM; Catalog #36150).
3. To each tube containing 1.7 mL of medium, add 0.3 mL of cytokines (prepared in step 2) to achieve a volume of 2 mL.

4. Prepare a cell suspension at 33X the desired final concentration in Iscove's MDM.

   NOTE: Suitable cell density is dependent on the cytokine combination used. Each researcher should establish the appropriate cell concentration for each application. Refer to the Technical Manual (Document #10000005592) for recommended plating concentrations.
5. To each tube containing 2 mL of medium with cytokines, add 0.1 mL of the cell suspension (prepared in step 4).
6. Mix one tube of medium containing cells (2.1 mL total volume).
7. Using a sterile 2 mL serological pipette (e.g. Catalog #38002), transfer 1.2 mL of cold Collagen Solution to the tube. Pipette up and down to mix.
8. Using the same 2 mL pipette, remove 1.5 mL of the final culture mixture and dispense 0.75 mL into each of the 2 wells of a previously labeled MegaCult™-C Double Chamber Slide (Catalog #04813).
9. Dispense another 1.5 mL in the same manner onto a second chamber slide. Remove any air bubbles by gently touching the bubble with the end of the pipette.

   NOTE: If more than one tube is being used, Collagen Solution should be added to the first tube only, and the contents dispensed into chamber slides before proceeding to the next tube.

   NOTE: Chamber slides should be labeled with a pencil or diamond point pen. Ink labeling will become illegible during the fixation process.
10. Gently tip each slide using a circular motion to allow the mixture in the chambers to spread evenly over the surface of the slide.
11. Place each slide in a 100 mm culture dish (e.g. Catalog #38045) containing an open 35 mm culture dish (e.g. Catalog #27100/38069) with 3 mL of sterile water to maintain optimal humidity during the incubation period. Replace lid of 100 mm dish.
12. Transfer the slides to a 37°C incubator with an atmosphere of 5% CO_2 and ≥ 95% humidity. Gel formation will occur within approximately 1 hour. It is important not to disturb the cultures during this time.
13. Incubate for 10 - 12 days. Maximum CFU-Mk colony size and numbers are typically seen at this time. The slides are now ready for fixation and staining. Cultures should be visually assessed for overall colony growth and morphology using an inverted microscope prior to fixation and staining.