MegaCult™-C Medium with Cytokines

For Assays of Human Megakaryocyte Progenitors

Catalog #04901	2 mL x 24 tubes
Catalog #04951	50 mL

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Product Description

MegaCult™-C Medium with Cytokines is optimized for the detection and quantification of human megakaryocyte progenitors (CFU-Mk) in bone marrow (BM), mobilized peripheral blood (MPB) and cord blood (CB). It is suitable for CD34+ enriched cells, mononuclear cells and cells isolated by other purification methods.

The MegaCult™-C Collagen and Medium with Cytokines kit (Catalog #04961 [24 tubes] or Catalog #04965 [50 mL bottle]) includes medium and collagen solution for optimal growth of CFU-Mk.

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)
- 2-Mercaptoethanol
- rh Thrombopoietin
- rh IL-6
- rh IL-3
- Supplements

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.

Handling / Directions for Use

For complete instructions for the assay of human CFU-Mk refer to the Technical Manual: MegaCult™-C Assay for Quantitation of Human and Mouse Megakaryocytic Progenitors (Document #28413), available on our website 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 Cytokines at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix well.
2. Aliquot the desired volume into tubes (e.g. 2 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 Cytokines at room temperature (15 - 25°C) or overnight at 2 - 8°C. Place thawed medium and Collagen Solution (Catalog #04902) on ice.

NOTE: If not used immediately, store tubes of MegaCult™-C Medium with Cytokines at 2 - 8°C for up to 2 weeks.

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

NOTE: Refer to the Technical Manual (Document #28413) for recommended plating concentrations.

3. To each tube containing 2 mL of MegaCult™-C Medium with Cytokines add 0.1 mL of the cell suspension (prepared in step 2).
4. Vortex tube of medium containing cells (2.1 mL total).

5. Using a sterile 2 mL pipette, transfer 1.2 mL of cold Collagen Solution (Catalog #04902) to the tube. Pipette up and down to mix.

6. 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).

7. 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.

8. Gently tip each slide using a circular motion to allow the mixture in the chambers to spread evenly over the surface of the slide.

9. Place each slide in a 100 mm Petri Dish (Catalog #27110) containing an open 35 mm Culture Dish (Catalog #27100) with 3 mL of sterile water to maintain optimal humidity during the incubation period.

10. 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.

11. 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.