1201 Mouse Base Medium

Methylcellulose-Based Medium for CFC Assays

Catalog #	Unit Size (cells/vial)
1201	90 mL bottle

Product Description

ColonyGEL™ 1150 is a methylcellulose-based medium containing specially selected PHA-Leukocyte Conditioned Medium as a source of growth factors to promote the growth of leukemic colonies from multiple myeloma patient bone marrow (BM).

The MM-CFC assay is an in vitro quantitative assay used to determine the frequency of multiple myeloma progenitors based on their ability to form unique, morphologically distinctive colonies in semi-solid media.

ColonyGEL™ 1150 has been formulated for the growth and evaluation of multiple myeloma leukemic colonies in the MM-CFC assay.

Confirmatory Assay: pluck colonies and evaluate cell surface phenotype by FACS (MM is CD138+).

Stability and Storage

Store the entire bottle of media or aliquots at -20°C. The product is stable at -20°C for up to 2 years from the date of manufacture. Storage at 4-8°C is NOT recommended.

If the product is received partially thawed, immediately place the product at -20°C or allow the product to thaw for aliquoting into working volumes (please refer to the back of this sheet for proper procedure).

Product Formulation

Methylcellulose in IMDM
Pre-screened Fetal Bovine Serum
Pre-screened Bovine Serum Albumin
L-Glutamine
2-Mercaptoethanol
Recombinant Human Insulin
Human Transferrin

Limited Product Warranty

ReachBio LLC warrants only that these products will perform according to established product specifications and makes no warranty as to their utility or fitness for use for any application whatsoever. Seller provides the products to the purchaser with the understanding that the purchaser is solely responsible for determining if the product is suitable for his or her intended application. Seller shall not be liable for any damages or injury to persons or property, arising from the purchase or use of the product, or for any results or failure to obtain results arising out of the use of the products. In addition, Seller shall not be liable for the product after the product expiration date or if the product has been misused, damaged or has otherwise become unusable due to improper storage or handling by purchaser. This warranty is exclusive and limits Seller's liability to the replacement of the product or, at Seller's option, the full credit of the original purchase price.

NO OTHER WARRANTIES OF ANY KIND, EXPRESSED OR IMPLIED, INCLUDING WITHOUT LIMITATION, IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE ARE PROVIDED BY SELLER, AND SELLER SHALL HAVE NO LIABILITY FOR ANY DIRECT, INDIRECT, CONSEQUENTIAL OR INCIDENTAL DAMAGES ARISING OUT OF THE USE, THE RESULTS OF USE, OR THE INABILITY TO USE ANY PRODUCT.

THIS PRODUCT IS FOR IN VITRO RESEARCH USE ONLY

This product is NOT intended or approved for human or veterinary use, or for use in clinical diagnostic or therapeutic procedures.

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Table 1. Recommended Plating Concentrations[1]		
Cell Source**	Cells per 35 mm Dish	Cell Concentration (cells / mL)
BM (Untreated)	2×10^4	2×10^5
Spleen (NH_4Cl)	2×10^5	2×10^6
PB (NH_4Cl Treated)	3×10^4	3×10^6
Day 14 FL	2×10^4	2×10^5

**Note: Plating concentrations may vary depending on the mouse strain used.

Procedure for Use – Addition of Cytokines Prior to Aliquoting Media

1. Add 10 mL of the desired cytokine to the 90 mL bottle of ColonyGEL™ Base Medium, shake the bottle vigorously for 30 seconds to ensure equal distribution of contents and let stand for 10 minutes (max. 20 minutes) before aliquoting into 4.0 mL or 3.0 mL aliquots for triplicate or duplicate assays, respectively.
2. For Triplicate Assays: aliquot 4.0 mL of medium by positive displacement into individual tubes. To do this, insert a 10 mL syringe attached to a 16 gauge blunt end needle (Catalog #3011) into the bottle and draw up approximately 1.0 mL of medium. Dispense this back into the original bottle to eliminate air bubbles from the syringe. Repeat if necessary.
3. Draw up 9.0 mL of medium into the syringe and dispense 4.0 mL into an appropriately labeled tube (there is now 5mL of medium remaining in the syringe or the medium is now at the 5.0 mL mark). Dispense another 4.0 mL into a second tube (the medium is now at the 1.0 mL mark).
4. Using the same syringe, draw up another 8.0 mL of medium (there should still be 1.0 mL of medium in the syringe) and dispense 4.0 mL aliquots into separate tubes as indicated in Step 4. Repeat the procedure until the entire 100 mL of medium has been aliquoted.
5. Store each 4.0 mL aliquot of medium at -20°C.

*Note: if only duplicate cultures are required, eliminate the air bubble from the syringe as described in Step 3, then draw up the medium to the 10.0 mL mark. Dispense 3.0 mL into the first tube (the medium is now at the 7.0 mL mark), dispense 3.0 mL into a second tube (the medium is now at the 4.0 mL mark) and dispense 3.0 mL medium into a third tube (the medium is now at the 1.0 mL mark). Draw up another 9.0 mL of medium and continue to dispense 3.0 mL aliquots into separate tubes. Repeat this procedure until the entire 100 mL of medium has been aliquoted.

Aliquoting Media Prior to the Addition of Cytokines

1. Aliquot the 90 mL of ColonyGEL™ Base Medium into 3.6 mL or 2.7 mL aliquots for triplicate or duplicate assays before adding the desired cytokine combination.
2. For Triplicate Assays: aliquot 3.6 mL of medium by positive displacement into individual tubes. To do this, insert a 10 mL syringe attached to a 16 gauge blunt end needle (Catalog #3011) into the bottle and draw up approximately 1.0 mL of medium. Dispense this back into the original bottle to eliminate air bubbles from the syringe. Repeat if necessary.

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3. Draw up 9.0 mL of medium into the syringe and dispense 3.6 mL into an appropriately labeled tube (there is now 5.4 mL of medium remaining in the syringe or the medium is now at the 5.4 mL mark). Dispense another 1.8 mL into a second tube (the medium is now at the 1.8 mL mark).
4. Using the same syringe, draw up another 7.2 mL of medium (there should still be 1.8 mL of medium in the syringe) and dispense 3.6 mL aliquots into separate tubes as indicated in Step 3. Repeat the procedure until the entire 90 mL of medium has been aliquoted.
5. Store each 3.6 mL aliquot of medium at -20°C.
6. Prior to use, add 400 uL (for triplicate assays) or 300 uL (for duplicate assays) of the desired cytokine combination after thawing the media (see Plating Cells in ColonyGEL™ Medium).

*Note: if only duplicate cultures are required, eliminate the air bubble from the syringe as described in Step 3, then draw up the medium to the 10.0 mL mark. Dispense 2.7 mL into the first tube (the medium is now at the 7.3 mL mark), dispense 2.7 mL into a second tube (the medium is now at the 4.6 mL mark) and dispense 2.7 mL medium into a third tube (the medium is now at the 1.9 mL mark). Draw up another 7.1 mL of medium and continue to dispense 2.7 mL aliquots into separate tubes. Repeat this procedure until the entire 90 mL of medium has been aliquoted.

Plating Cells in ColonyGEL™ Medium

1. Thaw the appropriate number of tubes of medium overnight at 4°C or at room temperature.
2. Add desired cytokine combination if not already added: 400 uL (for triplicate assays) or 300 uL (for duplicate assays) of desired cytokine combination to each 3.6 mL or 2.7 mL of base medium, respectively.
3. Prepare the cells required for the assay to a solution 10 fold higher than the desired concentration in the assay (Table 1).
4. To a 4.0 mL tube (triplicate assays) of ColonyGEL™ medium, add 400 uL of the cells; to a 3.0 mL tube (duplicate assays) of ColonyGEL™ medium add 300 uL of cells. ***Ensure that the appropriate cytokines have been added to the Base Medium.*** Vortex the tubes containing the medium, cytokines and cells (mixture) for a minimum of 5 seconds and let stand for 2 minutes to allow bubbles to rise to the top before plating.
5. Using a 3 cc syringe and a 16 gauge blunt end needle (Catalog #3011), draw up a small amount of the mixture (methylcellulose containing cells) and dispense back into the original tube. This serves to remove the air bubbles within the syringe. Plate 1.1 mL of medium containing cells into three (for triplicate assays) or two (for duplicate assays) 35 mm dishes by positive displacement as described in Step 6 below.
6. Draw up 2.6 mL of the mixture and dispense 1.1 mL into one of three (or two) labeled 35 mm dishes. Dispense another 1.1 mL into a second 35 mm dish (the mixture is now at the 0.4 mL mark of the syringe).
7. If performing triplicate assays, draw up another 1.1 mL of the mixture (1.5 mL mark of the syringe) and dispense 1.1 mL into the final 35 mm dish (this step is not required for duplicate assays).
8. Swirl the semi-solid mixture in the 35 mm dishes until the bottom of the dishes have been completely covered. Place the 35 mm dishes containing the medium and cells into a 100 mm or 245 mm dish that has a cover.
9. Add two additional 35 mm dishes, each containing 2 mL of water (without lids), into the same 100 mm or 245 mm dish and cover. The two 35 mm dishes containing water serve to ensure a good humidity for the duration of the cultures.
10. Place the cultures in a water jacketed incubator at 37 +/- 0.5°C, 5 +/- 0.5% CO_2 for 14-16 days.

1. Reference: Periera et. al. (2007). Hematopoietic Colony-Forming Cell Assays. In Vemuri (Ed.), Methods in Molecular Biology, vol. 407: Stem Cell Assays (pp. 177-208). New Jersey: Humana Press.

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