1100 Stock Methylcellulose Medium

Methylcellulose-Based Medium for CFC Assays

Catalog #	Unit Size (cells/vial)
1100	100 mL bottle

Product Description

ColonyGEL™ 1100 is a stock methylcellulose medium in IMDM. The base medium is designed to allow individual researchers to customize the medium for colony-forming cell (CFC) assays by adding their own cytokines and other supplements specific to their research. Depending on the cytokine combination and supplements added, this medium can support the growth of myeloid, erythroid and mixed lineage progenitor-derived colonies such as CFU-GM, CFU-G, CFU-M, BFU-E, CFU-E, CFU-GEMM from human peripheral blood (PB), bone marrow (BM), cord blood (CB), and mobilized peripheral blood (MPB). Mouse, rat, non-human primate and canine cells can also be used with the addition of species-specific cytokines and supplements.

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

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

IMDM

Please contact ReachBio Research Labs technical support at 206.420.0300 or techsupport@reachbio.com with questions concerning the formulation of this product.

Limited Product Warranty

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.

ReachBio Research Labs 123 NW 36th St., Ste. 235, Seattle, WA 98107
T 206.420.0300 | **F** 206.420.0301 | **TF** 877.838.CELL(2355) | **E** techsupport@reachbio.com

Procedure for Use – Preparing Aliquots of ColonyGEL™ Stock Medium

The 100 mL of ColonyGEL™ 1100 Stock Methylcellulose Medium needs to be aliquoted into working volumes for making up custom formulations. Due to the viscosity of the medium, it is recommended that each working volume of stock medium is at least 10 mL. Every 10 mL of ColonyGEL™ 1100 Stock Methylcellulose Medium is sufficient to make up a total of 21.5 mL of your own complete custom medium (i.e. you must add 11.5 mL of supplements and cytokine combination). In order to maintain the correct viscosity of the medium and the correct concentration of methylcellulose for CFC assays, the 10 mL stock medium must be made up to a total of 21.5 mL of your complete custom medium.

1. Thaw the 100 mL bottle of ColonyGEL™ Stock Methylcellulose Medium overnight at 4°C or at room temperature.
2. Using a 16 gauge, blunt-end needle and syringe (Catalog #3011), aliquot the 100 mL of medium into 10 mL working aliquots. Each aliquot can be stored at -20°C until use.

Note: Use a 25 mL bottle for each 10 mL aliquot since the final volume of the custom complete media (once made up) will be 21.5 mL total. Due to the viscosity of the stock medium, there will be some medium loss.

Preparing Complete Custom Media from 10 mL Stock Methylcellulose Aliquots

1. Thaw each 10 mL bottle of ColonyGEL™ Stock Methylcellulose Medium at 4°C or at room temperature.
2. Prepare the desired cytokines and supplements to a total volume of 11.5 mL and add this mixture to the 10 mL bottle of stock medium (total of 21.5 mL).
3. Shake or vortex the bottle vigorously to equal distribution of the medium, cytokines and supplements and let stand for 10 minutes or until the air bubbles have risen to the top of the bottle before aliquoting.
4. Aliquot the 21.5 mL of medium into 3 mL (for duplicate assays)* or 4 mL (for triplicate assays) working volumes. Each 3 mL or 4 mL aliquots of the complete custom medium can be stored at -20°C until use.
5. 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.
6. Draw up 9.0 mL of medium into the syringe and dispense 4.0 mL into an appropriately labeled tube (there is now 5.0 mL 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).
7. 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 5. Repeat the procedure until the entire 21.5 mL of medium has been aliquoted.
8. Store each 4.0 mL aliquot of complete custom medium at -20°C.

   * Note: If only **duplicate** cultures are required, eliminate the air bubble from the syringe as described in Step 5, 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 the last 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 21.5 mL of medium has been aliquoted.

Plating Cells in ColonyGEL™ Medium

1. Prepare the cells required for the assay to a solution 10 fold higher than the desired concentration in the assay.
2. To a 3.0 mL (duplicate assays) of ColonyGEL™ medium containing the appropriate cytokines and supplements (added in Step 2 under Preparing Custom Complete Medium from 10 mL Stock

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Methylcellulose Aliquots), add 300 uL of cells. To a 4.0 mL tube (triplicate assays) of ColonyGEL™ medium containing the appropriate cytokines, add 400 uL of cells.

3. Vortex the tubes containing the ColonyGEL™ medium 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.
4. Using a 3 cc syringe and a 16 gauge blunt end needle (Catalog #3011), draw up a small amount of the mixture and dispense back into the original tube. This serves to remove the air bubbles within the syringe. Plate 1.1 mL of the mixture into two or three 35 mm dishes by positive displacement.
5. **For Triplicate Assays:** Draw up 2.6 mL of the mixture and dispense 1.1 mL into one of three 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). Draw up another 1.1 mL of the mixture (1.5 mL mark of the syringe) and dispense 1.1 mL into the third and final 35 mm dish.

Note: If only **duplicate** cultures are required, draw up 2.6 mL of the mixture containing cells and the desired cytokines and dispense 1.1 mL into two labeled 35 mm dishes. The remainder of the mixture can be discarded.

6. 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 ColonyGEL™ medium and cells into a 100 mm or 245 mm dish that has a cover.
7. 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. *Note: do not cover the water dishes.*
8. Place the cultures in a water jacketed incubator at 37 +/- 0.5°C, 5 +/- 0.5% CO_2 for the appropriate number of days depending on the assay and species.

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