

## PRODUCT DESCRIPTION:

This product contains the following components:

### CollagenCult® Medium without Cytokines

Catalog #04700  
1.7 mL/tube, 24 tubes/rack

### Collagen Solution

Catalog #04902  
35 mL/bottle

CollagenCult® is recommended for the assay of human hematopoietic progenitor cells from bone marrow, mobilized peripheral blood, cord blood, and CD34<sup>+</sup> enriched samples. This product is also suitable for the detection of human BFU-E, CFU-GM, and CFU-GEMM, with the addition of recombinant cytokines, and the detection of mouse CFU-GM with the addition of recombinant cytokines. Refer to Table 2 for recommended cytokine combinations.

## FORMULATION:


Components in final complete medium include:

Collagen  
Fetal Bovine Serum  
Bovine Serum Albumin  
rh Insulin  
Human Transferrin (Iron Saturated)  
2-Mercaptoethanol  
L-glutamine  
Iscove's MDM

**Note:** Addition of cytokines is required. Refer to Table 2 for recommended cytokine combinations for detection of BFU-E, CFU-GM, and CFU-GEMM.

*Human transferrin used in this product has been derived from human plasma. Venous blood from each donor has been tested for hepatitis B surface antigen (HBsAg) and HIV-1 antibody and/or HIV-1 antigen. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.*

Product Information Sheet



CollagenCult®  
Medium  
without  
Cytokines

Catalog #04742

Version 1.1.0

### CollagenCult® Medium without Cytokines

Catalog #04700  
1.7 mL/tube, 24 tubes/rack

Store CollagenCult® Medium at -20°C. Stable for at least 2 years at -20°C. Thaw individual tubes at room temperature or overnight under refrigeration (2-8°C). Do not thaw and freeze medium more than twice. Stable for two weeks when stored at 2-8°C.

### Collagen Solution

Catalog #04902  
35 mL/bottle

This product is stable for at least one year when stored at 2 - 8°C. The collagen solution at pH 2.0 (as supplied) will denature at temperatures greater than 26°C.

Freezing is not recommended.

CollagenCult® Medium (Catalog #04700) and Collagen Solution (#04902) are filter sterilized and each batch is sterility tested. Contents guaranteed sterile if seal is not tampered with.

These products are biological reagents, and as such, cannot be completely characterized or qualified. Some variation is unavoidable.

Products are performance tested using human hematopoietic cells in a hematopoietic colony-forming cell assay, and compared to a laboratory standard.

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## CULTURE PROCEDURE:

### Reagents and supplies:

- Double Chamber Slides (#04963)  
OR  
Culture Dishes and Slides (#04863)
- Cytokines (see Table 2)
- Culture medium for cell washing and dilution (e.g. Iscove's MDM, #36150)
- Fixation and staining solutions

**Note:** *CollagenCult<sup>®</sup> Medium (#04742) and either set of slides (#04963 or #04863) are sufficient for 24 duplicate assays (2 slides for each assay) or 48 single assays (1 slide for each assay).*

### Equipment:

- Certified biohazard containment hood for Level II handling
- Water jacketed 37°C incubator with 5% CO<sub>2</sub> in air and ≥95% humidity
- Light microscope with 5X and 10X objectives

### Procedure:

1. Thaw tubes of medium at room temperature or overnight under refrigeration (2-8°C) and place medium and collagen solution on ice.
2. Add the desired cytokines and Iscove's MDM (in 0.3 mL) to the medium without cytokines (1.7mL/tube) to achieve a final volume of 2.0 mL of complete medium. See **Table 2** for suggested cytokine combinations.
3. Prepare cell suspension in Iscove's MDM at 33X the desired final cell concentration. See **Table 1** for suggested plating concentrations.
4. Add 0.1 mL of the cell suspension to each tube containing 2.0 mL of the complete medium with cytokines (prepared in step 2).
5. Vortex tube of medium containing cells (2.1 mL total volume). Using a sterile 2 mL pipette, transfer 1.2 mL of cold collagen solution to the tube and vortex again (3.3 mL final volume). Using the same 2 mL pipette, remove 1.5 mL of the final culture mixture and dispense 0.75 mL into each of the two wells of a previously labeled double chamber slide, or 1.5 mL per 35 mm dish. Dispense another 1.5 mL in the same manner into a second double chamber slide or dish. Remove any air bubbles by gently touching bubble with the end of the pipette.

*The collagen starts to gel within several minutes following the addition to the cell suspension. If more than one tube is being setup, collagen should be added to the first tube only, and the contents dispensed into chamber slides or dishes before proceeding to the next tube.*

6. Gently tip each slide/dish using a circular motion to allow the mixture to spread evenly over the surface.
7. Place each slide or two 35 mm dishes in a 100 mm petri dish containing an open 35 mm petri dish with 3 mL of sterile water to maintain optimal humidity during the incubation period. Replace the lid of the 100 mm petri dish. Slides can also be placed in a covered 245 mm square bioassay dish with 2 or 3 open 35 mm petri dishes containing sterile water.
8. Transfer the slides/dishes to an incubator set at 37°C, 5% CO<sub>2</sub>, and ≥95% humidity. *Gel formation will occur within approximately one hour. It is important not to disturb the cultures during this time.*
9. Culture human progenitor assays for 14 - 16 days and mouse progenitor assays for 12 days. Maximum colony size and number 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.

### Notes:

1. A 3.3 mL volume allows two double chamber slides (4 x 0.75 mL cultures) or two 35 mm dishes (2 x 1.5 mL cultures) to be set up.
2. It is possible to split the complete medium containing cytokines into two tubes (2 x 1.0 mL) in order to carry out more tests with different samples, rather than duplicate assays with the same sample. In this case, the volume of cells and amount of collagen must be reduced accordingly (e.g. add 0.05 mL cells and 0.6 mL collagen to 1 mL medium).
3. Slides should be labeled with a pencil or diamond point pen. Ink labelling will become illegible during the fixation process.

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## DEHYDRATING AND FIXING PROCEDURE:

**Important:** The optimal dehydration and fixation procedures will vary with the staining system used, and must be established by the researcher.

### Materials:

- Acetone (use high quality grade suitable for fixation of cells)
- Filter cards (provided with #04963 and #04863)
- Spacers (provided with #04963 and #04863)
- Glass or plastic 2.5 L container with a tight fitting lid (ensure container is resistant to acetone solvent)
- Ice container

### Procedure:

1. Place 200 mL of acetone in a 2.5 L container and tightly seal the lid to prevent evaporation of the fixative. Place on ice for a minimum of 15 minutes.

*It is important to use a high quality grade of acetone.*

2. Leave the cultures at 37°C until just prior to dehydration as the collagen gel may become unstable at lower temperatures. Remove slides from the incubator individually or in small batches.

*Ensure that each slide is correctly labeled with a pencil or diamond point pen. Ink labeling will become illegible when the slide is immersed in the fixative solution.*

3. **a) If double chamber slides are used:** Carefully remove the plastic walls and rubber seal of the chamber slide without damaging the culture using the following procedure:

- Partially remove the plastic chamber by pulling it up and away from the slide (use forceps to hold the slide down) starting at one corner near the labeled end of the slide.
- When the rubber seal surrounding the collagen gel is visible under the plastic chamber, use forceps to grasp one corner of the rubber seal and gently stretch it over the corner of the plastic chamber so that it remains hooked there.

- Use forceps to hook the rubber seal over the other corner of the plastic chamber at the same end of the slide.
- The plastic chamber and rubber seal can now be easily removed in one motion by continuing to slowly pull them up and away from the slide. Gel and associated liquid will remain on the slide.

### b) If 35 mm dishes are used:

Refer to the Product Information Sheet provided with Culture Dishes and Slides (#04863).

- Remove lid and release the gel from the walls of the petri dish by rimming with a thin plastic pipette tip or micro spatula.
  - Cover the petri dish with a pre-labeled glass slide.
  - While holding the petri dish firmly onto the glass slide, invert the dish and slide.
  - Gently shake and let the gel slip out of the petri dish and spread out evenly onto the glass slide. Remove the petri dish.
  - Gently remove folds in the gel using the tapered end of a micro spatula or pipette tip.
4. Gently place a spacer onto the slide, on top of the gel. Place a thick white filter card on top and allow the liquid to soak the card. **Do not apply pressure to the filter card.** The liquid should start to wick onto the filter card immediately. If it does not, **gently** apply a **small amount** of pressure to the corner of the filter card on the opposite end of the slide from the gel. *Applying too much pressure will lead to a grid pattern on the slides.*
  5. After the liquid has soaked the thick white card, remove the card leaving the spacer in place.
  6. Place the slides into the 2.5 L plastic container filled with 200 mL of acetone (container remains on ice during fixation). To ensure that the slides are completely covered with fixative, no more than 12 slides should be placed in the container at one time. Tightly seal the lid of the container. The spacer will float off, leaving two squares of gel on the chamber slide or one circle of gel on the large slides.  
*Acetone fixative should be changed after fixing every 12 slides.*

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7. Leave the slides in the acetone for 5 minutes on ice. *If the spacers do not float away from the slide, gently shake the container to see if they will detach.*  
*If a spacer remains adherent, remove that slide from the fixative solution and gently peel the gel from the spacer onto the slide using a micro spatula, being careful not to rip the gel. Working quickly before the fixative evaporates is key to the success of this process.*
8. Remove the slides from the fixative and allow to air dry, vertically.
9. Stain cultures immediately, or store at 2-8°C or -20°C in the dark for up to one month until staining can be performed.

## STAINING PROCEDURE:

### Materials:

- May-Grunwald staining solution
- Giemsa staining solution
- Distilled water

### Procedure:

1. Allow slides to come to room temperature before staining (approximately 5 minutes).  
*All steps of the staining procedure should be carried out with the slides in a horizontal position.*
2. Completely cover slides with the May-Grunwald staining solution and leave for 2 to 3 minutes. Rinse slides with cool tap water.
3. Completely cover the slides with a freshly prepared 1:20 dilution of Giemsa staining solution in distilled water (or prepared according to the manufacturer's instructions). Leave for 15 to 20 minutes, then rinse slides with cool tap water.
4. Allow the slides to air dry.  
*Stained slides should be stored in a covered container in a cool dry place.*

### Notes:

1. Ensure that the cultures are completely covered with staining solution.
2. At each step of the staining procedure, remove as much of the staining solution as possible by gently tipping the slide over a waste container and then carefully touching the edge of the slide to an absorbent paper.

## SCORING PROCEDURE:

First scan the entire slide using a 5X objective lens, noting the relative proximity of the colonies to each other. Scoring can then be performed with the same lens. Use a 10X objective to examine colonies in greater detail.

**BFU-E** tend to form tight colonies. Visible erythroblasts at the periphery of the colony have intense nuclear staining and minimal cytoplasm.

**CFU-GM** colonies are more dispersed and individual cells have a more diffuse nuclear staining and a greater proportion of cytoplasm (lower nuclear:cytoplasm ratio). Cells tend to be larger than erythroblasts.

**CFU-GEMM** typically appear as colonies containing erythroblasts and granulocyte and/or macrophage cells.

**Table 1. Recommended Plating Concentrations:**

Cells	Cell Suspension (cells/mL)	Cells per Slide (1.5 mL volume)
<b>For Human Cells</b>		
Ammonium Chloride Treated Bone Marrow	1.1 x 10 <sup>6</sup>	5 x 10 <sup>4</sup>
Light Density (≤1.077 g/cm <sup>3</sup> ) Bone Marrow	4.4 x 10 <sup>5</sup>	2 x 10 <sup>4</sup>
CD34 <sup>+</sup> -Enriched Cells*	1.1-3.3 x 10 <sup>4</sup>	500-1500
<b>For Mouse Cells</b>		
Bone Marrow	4.4 x 10 <sup>5</sup>	2 x 10 <sup>4</sup>
Lineage-Depleted Bone Marrow <sup>†</sup>	2.2 x 10 <sup>4</sup>	1000

\* CD34<sup>+</sup>-enriched cell suspensions from human bone marrow, cord blood, or mobilized peripheral blood can be obtained using StemSep<sup>®</sup> (#14056), EasySep<sup>®</sup> (#19056 or #19057), or RosetteSep<sup>®</sup> (#15026) Human Progenitor Cell Enrichment Kits, or EasySep<sup>®</sup> Human CD34 Positive Selection Kit (#18056).

<sup>†</sup> Lineage-depleted cells from mouse bone marrow can be obtained using StemSep<sup>®</sup> (#13056), EasySep<sup>®</sup> (#19756), or SpinSep<sup>®</sup> (#17056) Mouse Progenitor Cell Enrichment Kits. For further details on StemCell's cell separation products, please contact a Technical Representative or visit our website at: [www.stemcell.com/product\\_catalog/cell\\_separation.asp](http://www.stemcell.com/product_catalog/cell_separation.asp)

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**TABLE 2: Suggested Cytokine Combinations**

Colony-Forming Cell Type Detected	Cytokine Combination	Catalog #
<b>Human Cells*</b>		
BFU-E, CFU-GM, CFU-GEMM	10 ng/mL rh IL-3	02503
	10 ng/mL rh GM-CSF	02532
	10 ng/mL rh G-CSF	02615
	50 ng/mL rh SCF	02630
	3 U/mL rh Epo	02625
CFU-GM	10 ng/mL rh IL-3	02503
	10 ng/mL rh GM-CSF	02532
	10 ng/mL rh G-CSF	02615
	50 ng/mL rh SCF	02630
<b>Mouse Cells†</b>		
CFU-GM	10 ng/mL rm IL-3	02733
	10 ng/mL rh IL-6	02506
	50 ng/mL rm SCF	02731
	OR	
	10 ng/mL rm IL-3	02733
	10 ng/mL rm GM-CSF	02732
	50 ng/mL rm SCF	02735

\* Human bone marrow, cord blood, mobilized peripheral blood, or CD34<sup>+</sup>-enriched cells

† Mouse bone marrow or lineage-depleted bone marrow cells

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