

## RECOMMENDED FOR

Quantification of CFU-Hill Colonies from human peripheral blood. This product has been developed to enable quantification of CFU-Hill colonies from human peripheral blood (PB). Components of the CFU-Hill Liquid Medium Kit (Basal Medium and Supplements) have been pre-screened and performance tested in an established *in vitro* colony assay.<sup>1</sup>

## PRODUCT DESCRIPTION

The CFU-Hill Liquid Medium Kit includes:

Component	#05900	#05950
CFU-Hill Basal Medium	80 mL	5 x 80 mL
CFU-Hill Medium Supplements	20 mL	5 x 20 mL

The CFU-Hill Medium Supplements are a biological reagent, and as such cannot be completely characterized or quantified.

CFU-Hill Liquid Medium Kit is aseptically manufactured using tightly controlled processes and extensively pre-screened components.

Each lot of CFU-Hill basal medium and supplement is sterility tested. A Certificate of Analysis is available upon request.

This product does not contain antibiotics.

## STABILITY AND STORAGE

CFU-Hill Basal Medium is stable at 2 - 8°C until expiry date indicated on label.

CFU-Hill Medium Supplements are stable at -20°C until expiry date indicated on label.

CFU-Hill Liquid Medium (Basal Medium and Supplements combined) is stable at 2 - 8°C for one month.

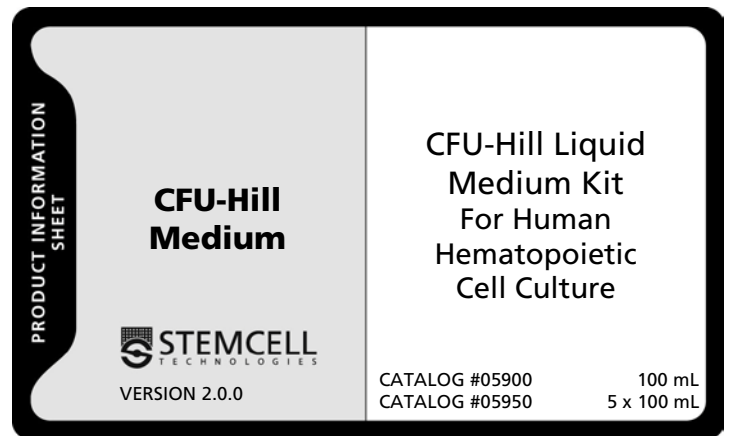
If product is received partially thawed, place immediately at -20°C or thaw and aliquot as described in 'Handling and Directions for Use'.

## HANDLING AND DIRECTIONS FOR USE

For complete instructions refer to the CFU-Hill Technical Manual (Manual; Catalog #28712) available on our website at [www.stemcell.com](http://www.stemcell.com).

### 1.0 Preparation of CFU-Hill Liquid Medium

- 1) Thaw CFU-Hill Medium Supplements under refrigeration (2 - 8°C) overnight or at room temperature.
- 2) Prepare CFU-Hill Liquid Medium by adding the entire volume of CFU-Hill Medium Supplements to the bottle of CFU-Hill Basal Medium, or add 2 mL of CFU-Hill Medium Supplements to every 8 mL of CFU-Hill Basal Medium (1/5 dilution). The CFU-Hill Liquid Medium will be stable at 2 - 8°C for one month.



- 3) Any thawed CFU-Hill Medium Supplements not used immediately should be dispensed into working aliquots and stored at -20°C. These aliquots can then be thawed as described in step 1 and stored at 2 - 8°C for no longer than one month. Avoid repeated freezing and thawing.
- 4) This product does not contain antibiotics. If desired, add Penicillin and Streptomycin Solution (Catalog #07500) to achieve a final concentration of 100 U/mL of Penicillin and 100 µg/mL of Streptomycin.

### 2.0 5 Day CFU-HILL Colony Assay

#### 2.1 Day 0

- 1) Collect PB aseptically using an anti-coagulant to avoid clotting or clumping of the sample. Sodium heparin, ACD (acid citrate dextrose), K<sub>3</sub> EDTA or equivalent may be used.  
*The protocol requires 10<sup>7</sup> mononuclear cells per sample for each experiment.*
- 2) Prepare a mononuclear cell suspension by light density separation using Ficoll-Paque™ PLUS (Catalog #07907/07917/07957/07967). For a more detailed protocol, recommended volumes, and appropriate tube sizes, consult the Ficoll-Paque™ PLUS product information sheet on our website ([www.stemcell.com](http://www.stemcell.com)). Briefly:
  - a) Add anti-coagulant treated PB to a 50 mL polypropylene tube (BD Catalog #352070 or equivalent). Add equal volume of Phosphate Buffered Saline (PBS; Catalog #37350) to the sample.
  - b) Add 15 mL of Ficoll-Paque™ PLUS to a new 50 mL polypropylene tube. Carefully layer the diluted blood sample on top of the 15 mL of Ficoll-Paque™ PLUS.  
*Do not mix the Ficoll-Paque™ PLUS and diluted blood sample.*
  - c) Centrifuge tube(s) at 300 x g for 25 minutes at room temperature with the brake "off."  
*See notes on the other side.*
  - d) After centrifugation, collect mononuclear cell layer into a new 50 mL polypropylene tube. Bring to a final volume of 40 mL with PBS + 2% fetal bovine serum (PBS + 2% FBS; Catalog #07905). Centrifuge at 300 x g for 7 minutes at

room temperature with the brake on to pellet the cell suspension.

- e) Remove supernatant and add 6 mL of PBS + 2% FBS to the tube. Resuspend the pellet and transfer cell suspension to a 15 mL centrifuge tube (Corning Catalog #430053 or equivalent). Rinse the 50 mL tube with an additional 6 mL of PBS + 2% FBS and transfer cell suspension to the same 15 mL tube. Centrifuge at 300 x g for 7 minutes at room temperature with the brake on to pellet the cell suspension.
- 3) Remove supernatant and resuspend pellet in 1 - 3 mL (depending on size of the cell pellet) of CFU-Hill Liquid Medium (refer to Section 1.0) and count nucleated cells using 3% Acetic Acid with Methylene Blue (Catalog #07060) at a 1/20 dilution (e.g. 10 µL sample in 190 µL acetic acid).
- 4) Add 2 mL/well of CFU-Hill Liquid Medium to a 6-well fibronectin-coated plate. Perform the experiment in duplicate using two wells of a 6-well plate per sample. Plate  $5 \times 10^6$  mononuclear cells per well and incubate for 2 days at 37°C, 5% CO<sub>2</sub> with ≥95% humidity.

*This step removes adherent cells.*

*Fibronectin-coated plates from BD BioSciences Discovery Labware are recommended; BD Catalog #354402, 6-well plates, 5/pack.*

Ficoll-Paque™ PLUS is a trademark of GE Healthcare LTD.

## 2.2 Day 2

After two days harvest the non-adherent cells containing the CFU-Hill colony-forming cells and culture for an additional three days to allow formation of CFU-Hill colonies.

- 1) Collect the non-adherent cells by pipetting the medium in each well up and down 3 - 4 times using a 2 mL pipette. This will help to remove any non-adherent cells that transiently attach to the adherent population. Transfer the non-adherent cells from each well into individual 5 mL tubes (BD Catalog #352058 or equivalent) and count nucleated cells using 3% Acetic Acid with Methylene Blue at a 1/10 dilution (e.g. 10 µL sample in 90 µL acetic acid).

*Do not wash or centrifuge the cells at this stage.*

- 2) From each well, plate  $1 \times 10^6$  cells/well in duplicate in a 24-well fibronectin-coated plate. Add fresh CFU-Hill Liquid Medium to a final volume of 1.0 mL per well.

*Fibronectin-coated plates from BD Biosciences Discover Labware are recommended; BD Catalog #354411, 24-well plates, 5/pack.*

- 3) Incubate at 37°C, 5% CO<sub>2</sub> with ≥ 95% humidity for 3 days.

## 2.3 Day 5

- 1) Count the number of CFU-Hill colonies per well for each sample. CFU-Hill colonies are defined as a central core of round cells with radiating elongated spindle-like cells at the periphery. For assistance with colony recognition and photographs of the cells at various stages of the protocol, please consult the "Atlas of CFU-Hill Colonies" (Catalog

#28711). Fixing and staining of colonies with Giemsa is recommended.

**Table 1. PB-Derived CFU-Hill colony frequencies in the general population**

Gender	Male (mean ± SD)	Female (mean ± SD)
Number of donors	15	13
Age Range	23 - 54	24 - 54
CFU-Hill number/10 <sup>6</sup> cells (range)	21 ± 18 (1 - 67)	20 ± 17 (2 - 58)
CFU-Hill number/mL of blood	35 ± 35	26 ± 23

## REFERENCES

- Hill JM, Zalos G, Halcox JP, *et al.*: Circulating endothelial progenitor cells, vascular function and cardiovascular risk. *N Engl J Med* 348:593-600, 2003

## NOTES

The CFU-Hill Liquid Medium Kit does not support the growth of mature endothelial cell lines, including human umbilical vein-endothelial cells (HUV-EC-C; ATCC # CRL-1730).

Please note that we changed the name of the EndoCult® Liquid Medium Kit to the CFU-Hill Liquid Medium Kit. This change was made to accurately reflect the application of this product. The formulation of the medium remains the same.

## Conversion of x g to rpm

To convert x g to rpm, use the following formula:

$$\text{RPM} = \sqrt{\frac{\text{RCF}}{(1.118 \times 10^{-5}) \times (\text{Radius})}}$$

Where: RCF = relative centrifugal force (g)  
RPM = centrifuge speed in revolutions per minute  
Radius = radius of rotor in cm

Please contact techsupport@STEMCELL.com if you have any questions or concerns.