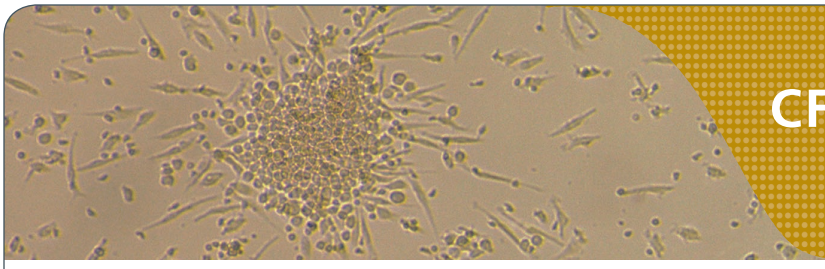


CFU-HILL LIQUID MEDIUM

ANGIOGENIC CELL RESEARCH

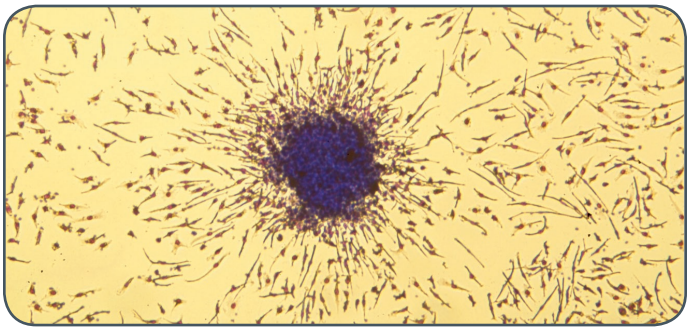
CFU-HILL COLONY ASSAY

Recently, several distinct populations of cells that appear to correlate with or influence postnatal cardiovascular health have been identified. Many of these may be referred to as “angiogenic cells”, i.e. cells that support or augment angiogenesis, without actually differentiating into the cells that form part of the vascular network.

A cell culture assay was developed by Hill *et al.*[1] to assess the correlation between the frequency of a specific population of circulating cells, clinical factors, and vascular function. In this widely used assay, peripheral blood mononuclear cells are plated on fibronectin-coated dishes. After 2 days, the non-adherent cells (which contain the cells of interest) are harvested and replated on fibronectin-coated dishes. Colonies, defined as a central core of “round” cells, with more elongated “sprouting” cells at the periphery, are evaluated and quantified 3 days later. In healthy individuals, the number of colonies negatively correlated with the Framingham cardiovascular risk score and positively correlated with vascular function, as measured by flow-mediated brachial artery reactivity.[1] STEMCELL Technologies has standardized this 5-day assay and refers to it as the 5-Day CFU-Hill Colony Assay. Unique colonies that are formed in the 5-Day CFU-Hill Colony Assay are referred to as Colony-Forming Unit - Hill Colonies or CFU-Hill Colonies. The CFU-Hill Liquid Medium Kit has been developed specifically to support the culture and quantification of CFU-Hill colonies. A growing list of associations between CFU-Hill colony frequency and various disease states, as described in the ‘CFU-Hill Colonies and Disease States’ section (below) highlights the utility of this assay.

CHARACTERIZATION OF THE CFU-HILL COLONY CELLS

Yoder *et al.*[2] showed that nearly all (>98%) of the cells generated in the 5-Day CFU-Hill Colony Assay expressed KDR (FLK1/ (CD309), CD14, and CD45, and some, but not all, expressed CD31, CD105 (endoglin), CD115 (M-CSF Receptor), CD144 (VE-Cadherin), CD146 (MUC-18/S-Endo), and von Willebrand Factor (vWF). Some cells in the colonies were able to bind the lectin UEA-1 and/or to take up acetylated low-density lipoprotein (Ac-LDL, whose incorporation is a hallmark of both endothelial cells and monocytes). The progeny of CFU-Hill colonies were able to ingest and kill microbes. Cells from CFU-Hill colonies could not be replated to form secondary CFU-Hill colonies. When replated in methylcellulose containing hematopoietic growth factors, they formed myeloid colonies. Taken together, these findings suggest that the cells comprising the CFU-Hill colonies are primarily monocytes and macrophages. However, the frequency of CFU-Hill colonies remains a potent biomarker of vascular health and correlates with many disease states, as described in the section ‘CFU-Hill Colonies and Disease States’ (next section).



Giemsa stained CFU-Hill colony

Recent studies have shown that monocytes and $CD4^+$ T cells are both necessary and sufficient to form CFU-Hill colonies.[3, 4] Cell-to-cell contact appears to activate the $CD4^+$ T cells to release soluble factors, which subsequently stimulate a subset of $CD14^+$ monocytes to form CFU-Hill colonies.[4] Gene products involved in inflammation or in supporting angiogenesis are upregulated in the cells of the CFU-Hill colony,[4] and soluble factors derived from the culture of CFU-Hill colonies support vascular network formation *in vitro*.[3] These data suggest that the cells that form the CFU-Hill colony have a role in vascular regeneration, and could be considered to be “angiogenic cells”.

CFU-HILL COLONIES AND DISEASE STATES

While CFU-Hill colonies may not include cells that can integrate into neovasculature, correlations between CFU-Hill colony frequency, cardiovascular risk factors, and cardiovascular function clearly show that the cells that form these colonies play some function in vascular homeostasis.[1] The CFU-Hill colony assay has, to date, been predominantly used to study vascular perturbations in disease states (i.e. as a biomarker of vascular homeostasis). A number of recent reports have used the 5-Day CFU-Hill Colony Assay in investigations into coronary artery disease,[5] peripheral arterial disease,[6] chronic obstructive pulmonary disease,[7] asthma[8] and acute ischemic stroke.[9]

WHY USE CFU-HILL LIQUID MEDIUM?

Recently, a number of reports have used the 5-Day CFU-Hill Colony Assay in investigations into colony frequency and:

- Coronary artery disease[5]
- Peripheral arterial disease[6]
- Chronic obstructive pulmonary disease[7]
- Asthma[8]
- Acute Ischemic stroke[9]

CFU-HILL LIQUID MEDIUM

ANGIOGENIC CELL RESEARCH

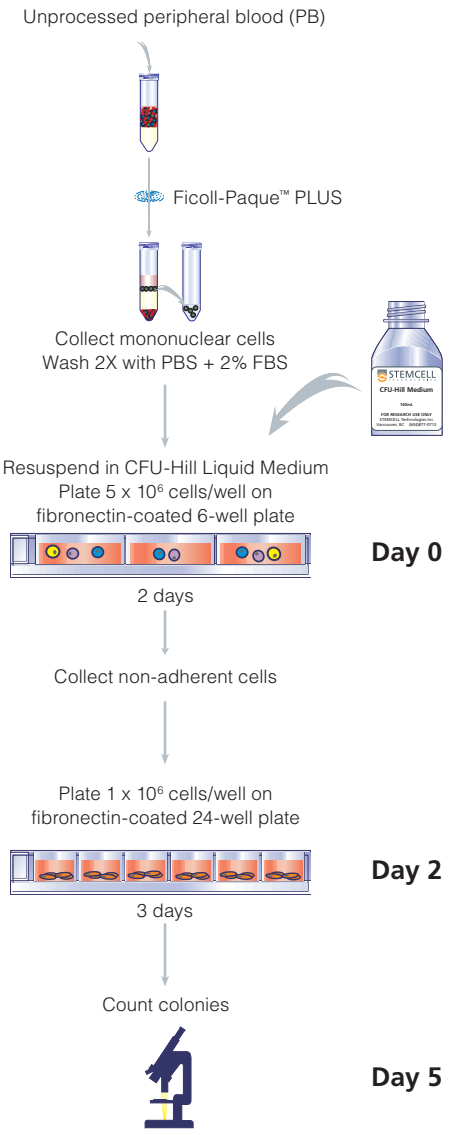
TABLE 1. CFU-Hill Product Information

PRODUCT NAME	APPLICATION	UNIT SIZE	CATALOG #
CFU-Hill Liquid Medium Kit	Basal medium and supplements for culture of CFU-Hill colonies from human peripheral blood	100 mL 500 mL	05900 05950
CFU-Hill Colony Atlas	Atlas of CFU-Hill colonies derived from human peripheral blood	1 atlas	28711
CFU-Hill Technical Manual	Complete information for culturing CFU-Hill colonies from human peripheral blood	1 manual	28712

TABLE 2. Human Peripheral Blood-derived CFU-Hill Colony Frequencies

GENDER	MALE (MEAN ± SD)	FEMALE (MEAN ± SD)
Number of donors	15	13
Age range	23 - 54	24 - 54
CFU-Hill number/10^6 cells (range)	21 ± 18 (1 - 67)	20 ± 17 (2 - 58)
CFU-Hill number/mL of blood	35 ± 35	26 ± 23

OUTLINE OF PROCEDURE. 5 Day CFU-Hill Colony Assay



The CFU-Hill Technical Manual is available at **www.stemcell.com**

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

REFERENCES

1. Hill JM, *et al.*, N Engl J Med 348: 593-600, 2003
2. Yoder MC, *et al.*, Blood 109: 1801-1809, 2007
3. Rohde E, *et al.*, Stem Cells 25: 1746-1752, 2007
4. Van Beem RT, *et al.*, J Immunol 180l: 5141-5148, 2008
5. Thum T, *et al.*, Am Coll Cardiol 46: 1693-1701, 2005
6. Shaffer RG, *et al.*, Cytometry B Clin Cytom 70: 56-62, 2006
7. Palange P, *et al.*, Eur Respir J 27: 529-541, 2006
8. Asosingh K, *et al.*, J Immunol 178: 6482-6494, 2007
9. Sobrino T, *et al.*, Stroke 38: 2759-2764, 2007

STEMCELL TECHNOLOGIES

THE CELL EXPERTS™ | WWW.STEMCELL.COM

IN NORTH AMERICA TOLL-FREE T. 1 800 667 0322 TOLL-FREE F. 1 800 567 2899
T. +1 604 877 0713 F. +1 604 877 0704 E. INFO@STEMCELL.COM

IN EUROPE TOLL-FREE T. 00 800 7836 2355 TOLL-FREE F. 00 800 7836 2300
T. +33 (0)4 76 04 75 30 F. +33 (0)4 76 18 99 63 E. INFO.EU@STEMCELL.COM

IN AUSTRALIA TOLL-FREE T. 1 800 060 350 F. +61 (03) 9338 4320 E. INFO.AUS@STEMCELL.COM

IN SINGAPORE T. +(65) 6776 7754 F. +(65) 6776 7114 E. INFO.SG@STEMCELL.COM

CATALOG #28567 | VERSION 3.0.0 | JUNE 2010