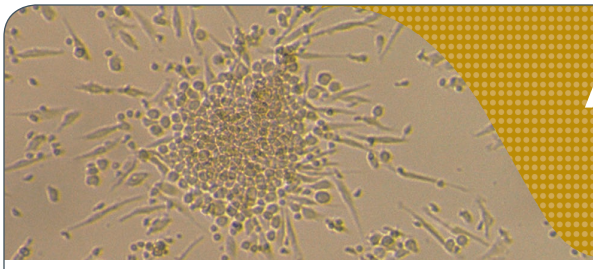


ATLAS OF CFU-HILL COLONIES

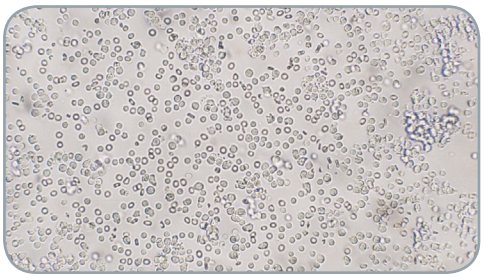
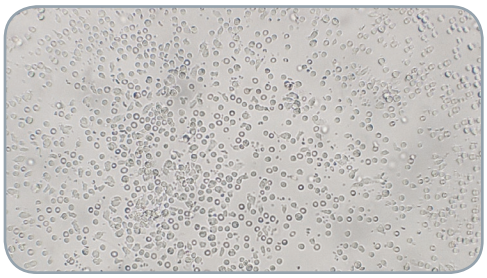
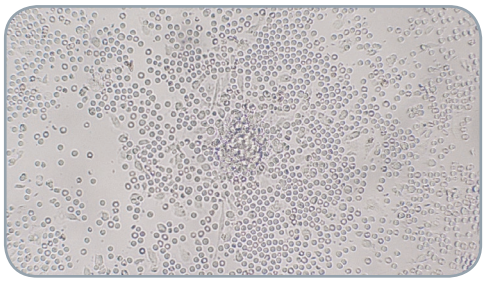
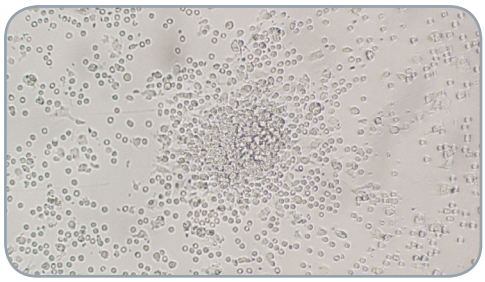
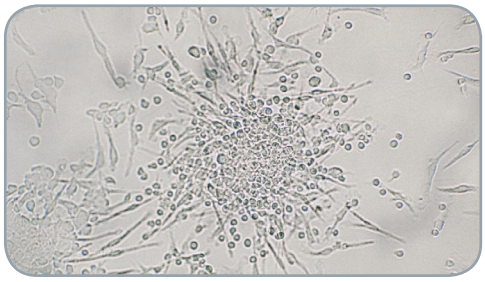
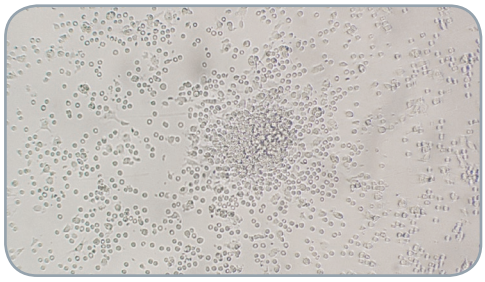
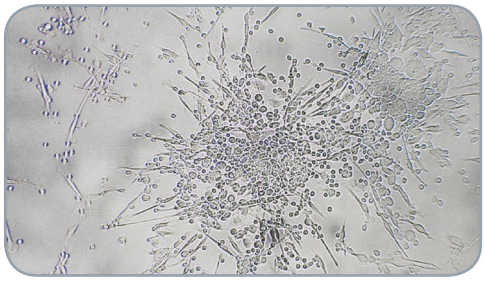
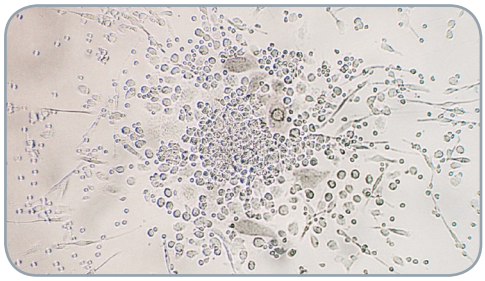
CULTURED WITH CFU-HILL LIQUID MEDIUM KIT

(CATALOG #05900/05950)

5 DAY CFU-HILL COLONY ASSAY

TIME COURSE OF COLONY FORMATION: Mononuclear cells from human peripheral blood are isolated by Ficoll-Paque™ PLUS density gradient centrifugation and plated on fibronectin-coated 6-well dishes in CFU-Hill Liquid Medium for two days to remove the adherent cell populations. After two days, the non-adherent cells are harvested and plated on fibronectin-coated 24-well plates. After 3 more days, colonies may form. CFU-Hill colonies are defined as those colonies that consist of two cell types: a central cluster of round cells and radiating elongated spindle-like cells at the periphery.* Colonies are evaluated at day 5, and can be fixed and stained for later quantification.

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

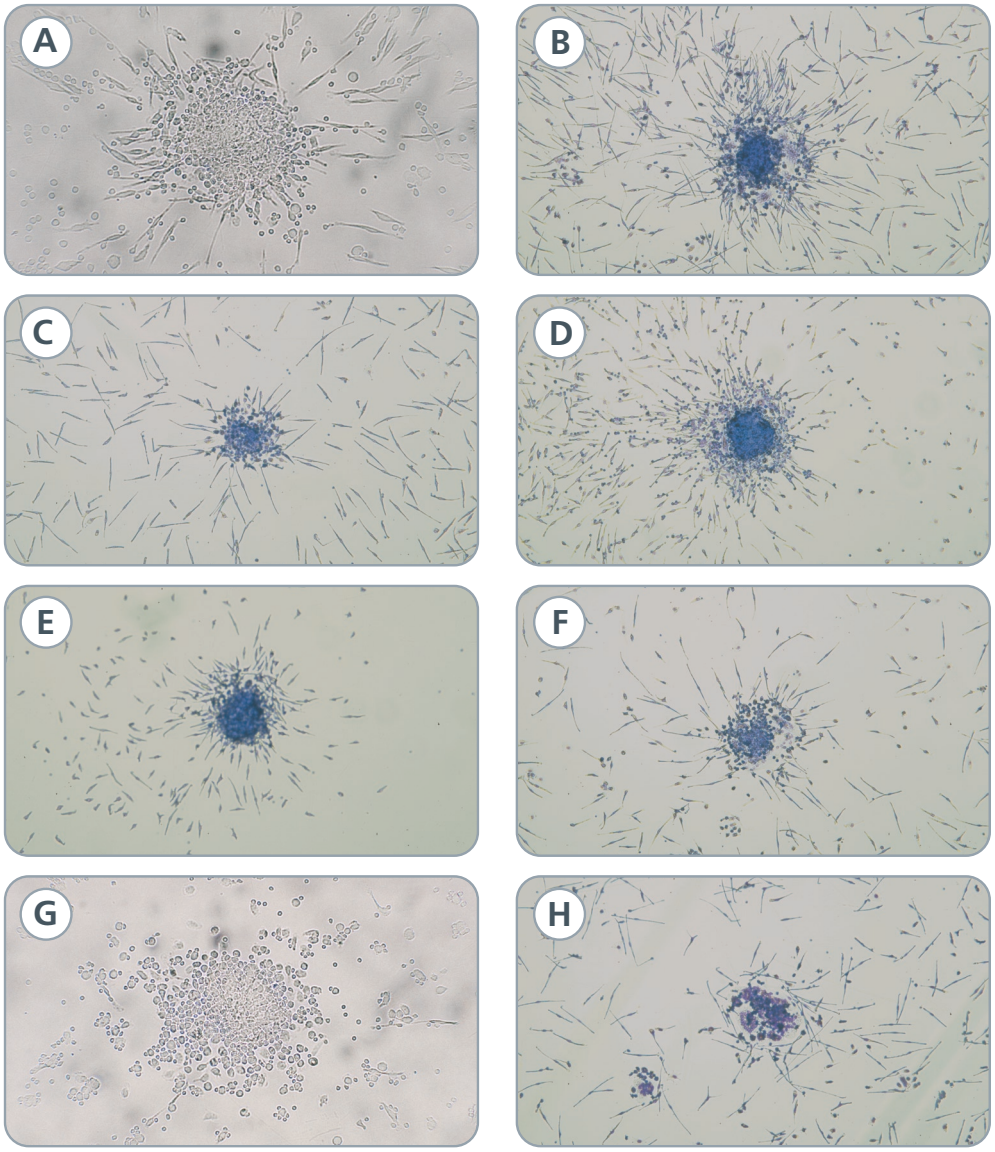
	DONOR 1	DONOR 2	
DAY 1			Cells appear viable. The majority are non-adherent cells which have a rounded morphology. The remaining adherent cells appear either as single cells or as small clumps (Magnification: 125X).
DAY 3			Small colonies may be visible. If present they may appear as rounded, ball-like clusters with few sprouts of elongated spindle-like cells at the periphery (Magnification: 125X).
DAY 4			Colonies are bigger in size with a more defined central cluster of round cells and more elongated spindle-like cells. Note the difference in size and morphology of the colonies between donors (Magnification: 125X).
DAY 5			Optimal time to evaluate and enumerate CFU-Hill colonies for most donors. Note: the central core of round cells may have a homogeneous morphology (Donor 1) or heterogeneous morphology (Donor 2). For Donor 2, optimal morphology appeared at day 4, but enumeration was still possible on day 5. With extended culture the morphology of colonies may alter and enumeration may become difficult (Magnification: 125X).

* Hill JM, Zalos G, Halcox JPJ, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T: Circulating endothelial progenitor cells, vascular function and cardiovascular risk. N Engl J Med 348:593-600, 2003

ATLAS OF CFU-HILL COLONIES

CULTURED WITH CFU-HILL LIQUID MEDIUM KIT

EXAMPLES OF CFU-HILL COLONIES AT DAY 5 OF CULTURE



PHOTOS A-F are of CFU-Hill colonies derived from human peripheral blood at day 5 of culture. All of these colonies are scored as CFU-Hill colonies and consist of two cell types: a central core of round cells, with radiating elongated spindle-like cells at the periphery. The spectrum of colony morphologies produced may vary among different donors in terms of size, the number of elongated spindle-like cells at the periphery, and the number of rounded cells in the center. Photo A is an unstained colony (Magnification: 125X). Photos B-F are colonies stained with Giemsa (Magnification : 50X).

PHOTOS G-H are of colonies derived from human peripheral blood at day 5 of culture that do not fulfill the criteria of CFU-Hill colonies. Photo G has only round cells. Photo H has both cell types, but the spindle-like cells are not in a radiating orientation. These are not scored as CFU-Hill colonies. Photo G is an unstained colony (Magnification: 125X). Photo H is a colony stained with Giemsa (Magnification: 50X).

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