STEMdiff™ Hematopoietic Kit

For the Generation of Hematopoietic Progenitor Cells from hPSCs

The **STEMdiff™ Hematopoietic Kit** consists of defined, serum-free basal medium and supplements designed for the generation of hematopoietic progenitor cells (HPCs) from human embryonic stem (hES) cells and induced pluripotent stem (hiPS) cells. Optimized for a standardized, 12-day differentiation protocol, this kit supports robust differentiation of human pluripotent stem cells (hPSCs) into HPCs that can be identified by the expression of CD34 and CD45 (Figures 1-3) and by the ability to form hematopoietic colonies of multiple lineages in colony-forming unit (CFU) assays with MethoCult™ medium (Figure 4). This kit is formulated for use in feeder-free conditions, optimized for the differentiation of hPSCs maintained in TeSR™ media and compatible with multiple hES and hiPS cell lines. After differentiation, the resulting HPCs may be used for additional downstream assays.

PRODUCT	SIZE	CATALOG #
STEMdiff™ Hematopoietic Kit*	1 Kit	05310
STEMdiff™ Hematopoietic Basal Medium	120 mL	05311
STEMdiff™ Hematopoietic Supplement A (200X)	225 µL	05312
STEMdiff™ Hematopoietic Supplement B (200X)	375 µL	05313

* Kit includes basal medium and supplements A and B.

Advantages:

DEFINED. Serum-free and feeder-free formulation.

EASY-TO-USE. Simple monolayer protocol produces HPCs in suspension for easy harvest.

RAPID. Generation of HPCs in 12 days.

HIGH YIELD. One kit typically generates 4 - 18 million $CD34^+CD45^+$ HPCs.

FLEXIBLE. Robust generation of HPCs across multiple hES and hiPS cell lines.

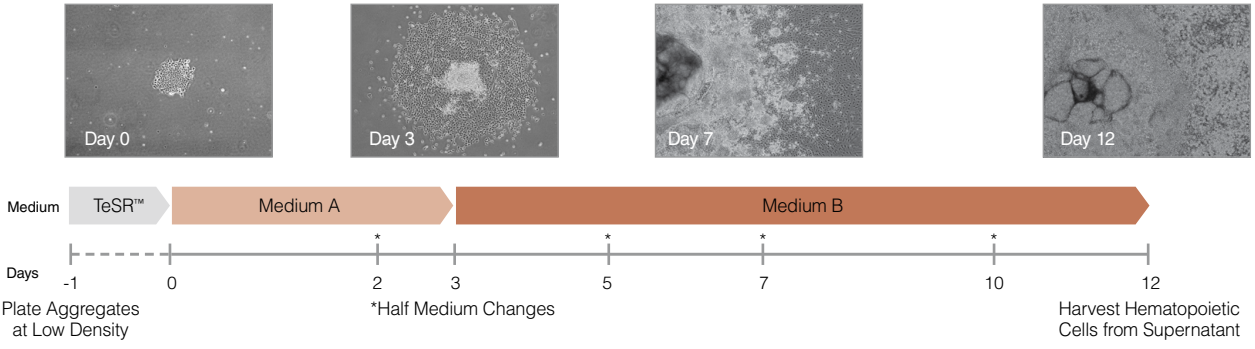

Figure 1. Hematopoietic Differentiation Protocol

One day before the differentiation protocol, hPSC colonies are harvested and seeded as small aggregates (100 - 200 µm in diameter) at 10 - 20 aggregates/cm^2 in mTeSR™1 or TeSR™-E8™. After one day, TeSR™ medium is replaced with Medium A (STEMdiff™ Hematopoietic Basal Medium containing Supplement A) to begin inducing the cells towards a mesoderm-like state (day 0). On day 2, a half medium change is performed with fresh Medium A. On day 3, the medium is changed to Medium B (STEMdiff™ Hematopoietic Basal Medium containing Supplement B) with half medium changes on days 5, 7 and 10, to promote further differentiation into hematopoietic cells. Typically, by day 12, large numbers of HPCs can be harvested from the culture supernatant.

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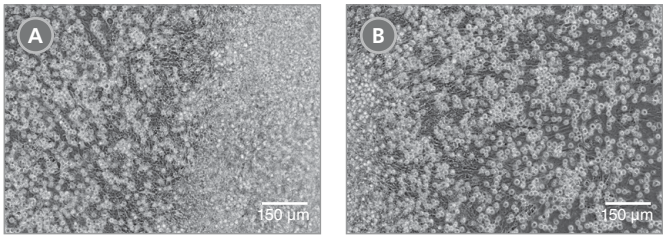

Figure 2. Morphology of hPSC-Derived HPCs

Representative images of (A) hES (H1) cells and (B) hiPS (WLS-1C) cells on day 12 of differentiation to HPCs using the STEMdiff™ Hematopoietic Kit. Differentiated cells exhibit typical HPC morphology as round cells that float freely in suspension.

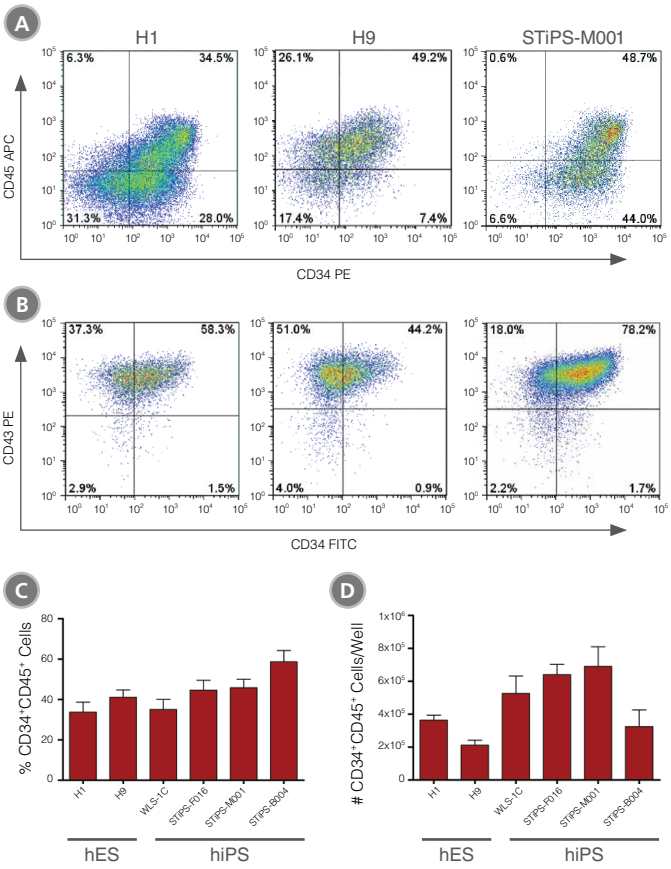

Figure 3. Efficient and Robust Generation of CD34⁺CD45⁺/CD43⁺ HPCs

hES and hiPS cells were cultured for 12 days in single wells of 12-well plates using the STEMdiff™ Hematopoietic Kit. At the end of the culture period, cells in suspension were harvested and analyzed by flow cytometry for expression of hematopoietic cell surface markers: CD34, CD45 and CD43. (A,B) Example flow cytometry plots for hematopoietic cell surface-marker analysis of cultures of hES (H1 and H9) and hiPS (STiPS-M001) cells. (C,D) Percentages and total numbers of CD34⁺CD45⁺ cells in cultures of hES (H1 and H9) or hiPS (WLS-1C, STiPS-F016, STiPS-M001 and STiPS-B004) cells are shown. Data shown as mean ± SEM; n ≥ 3.

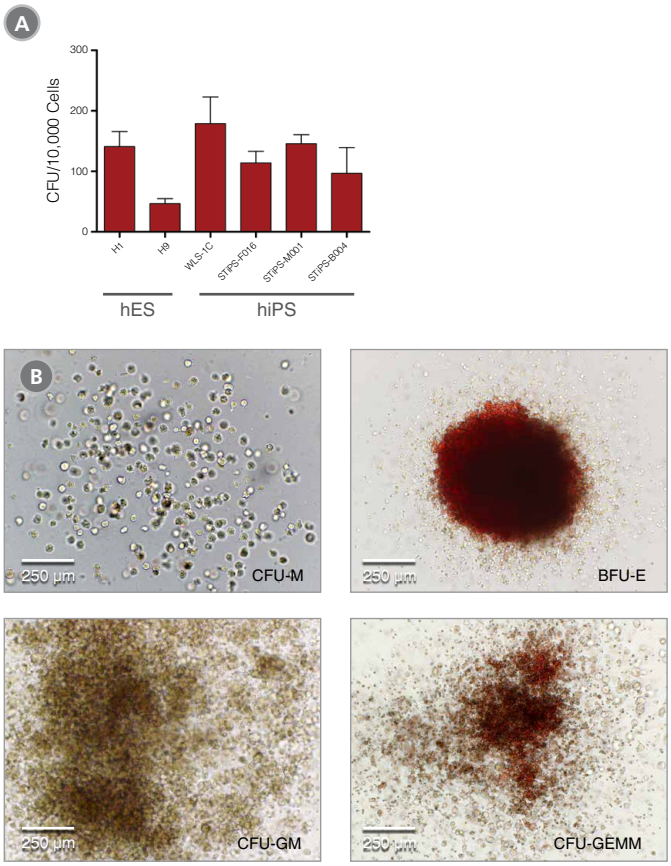

Figure 4. hPSC-Derived HPCs Produce Colonies of Multiple Lineages

Cells in suspension were harvested from cultures on day 12 of the hematopoietic differentiation protocol and assessed in colony-forming unit (CFU) assays using MethoCult™ H4435 Enriched (Catalog #04435) methylcellulose-based medium. Shown are (A) CFU frequencies observed in cultures of HPCs-derived from 6 different hPSC lines (data shown as mean ± SEM; n ≥ 3.) CFU frequencies were variable between different cell lines, with on average approximately 120 CFU per 10,000 hPSC-derived HPCs plated. The progenitor cell types observed included granulocyte/macrophage (CFU-M, CFU-G and CFU-GM), erythroid (BFU-E and CFU-E) and occasional mixed (CFU-GEMM) colonies. (B) A selection of representative colony images are shown at 40X magnification. MethoCult™ SF H4636 (Catalog #04636) has also been tested for use in CFU assays of hPSC-derived HPCs in serum-free conditions.

For a complete list of related products, including specialized cell culture and storage media, matrices, antibodies, cytokines and small molecules, visit **www.stemcell.com/hPSCworkflow** or contact us at **techsupport@stemcell.com**.