Data Sheet

HPC-7 Mouse Hematopoietic Precursor Cell Line

Cell Line

SCC466

Pack Size ≥ $1x10^6$ viable cells/vial

Store in liquid nitrogen

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Background

Hematopoietic stem cells (HSCs) are a type of stem cell that differentiate into all other mature blood cells derived from two lineages, myeloid and lymphoid. Hematopoiesis, a process in which HSCs differentiate into other mature blood cell lineages, occurs in the bone marrow, and gives HSCs unique regenerative properties in which they are capable of generating a variety of cell types as well as generating more stem cells in the process.[2]

HSCs migrate to injury sites and are useful for tissue repair. HSCs also appear to have potential for therapeutic treatment in inflammatory bowel disease models.[2] HSCs have already displayed some therapeutic benefit experimentally in models used for colitis and Crohn's disease.[3] Clinical use of HSCs through systemic injection has been quite limited by their rarity and poor tissue retention. Understanding HSC recruitment remains an important topic for improving stem cell-based therapies.

HPC-7, a mouse HSC cell line, was developed to further understand hematopoiesis and functionality of HSCs. The HPC-7 cell line retains many cell characteristics typical of fetal-derived hematopoietic precursors such as being c-kit+, CD34+, CD44+, Sca-1-, and Thy1.2-.[1] Transcription factors expressed by the HPC-7 cell line are also consistent with characterized HSCs. HPC-7 cells can be differentiated through the addition of different cell factors and have been shown to change cell-surface expression post-differentiation.[1] HPC-7 cells also retain the self-renewal properties characteristic of HSCs.

Source

- Genetically Modified Organisms (GMO).
- Mouse LH2 cDNA was retrovirally expressed in embryonic stem cells and subjected to differentiation assay *in vitro*. This led to the generation of multipotent hematopoietic precursor cells labeled HPC-7.[1]

Short Tandem Repeat

M18-3: 18	M6-7: 12	M1-2: 13	M7-1: 29	M1-1: 10	M3-2: 14	M8-1: 15.3
M2-1: 9	M15-3: 20.3	M6-4: 15.3	M11-2: 16	M17-2: 12	M12-1: 17	M5-5: 14
MX-1: 26	M4-2: 18.3, 19.3	M19-2: 12, 13	M13-1: 15.2, 16.2			

Quality Control Testing

- SCC466 cells are verified to be of mouse origin and negative for human, rat, Chinese hamster, Golden Syrian hamster, and non-human primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services
- Cells tested negative for infectious diseases against a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.

- Cells tested negative for mycoplasma.

Storage and Handling

SCC466 cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

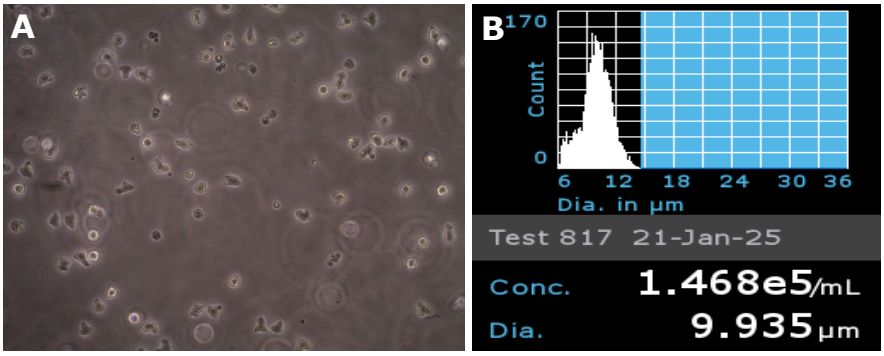

Figure 1. Bright-field image of SCC466 cells a day after thaw in a T25 flask (**A**). Cell counting was performed using Scepter™ 3.0 handheld automated cell counter using 60 µm sensor tips (**B**, MilliporeSigma PHCC360KIT).

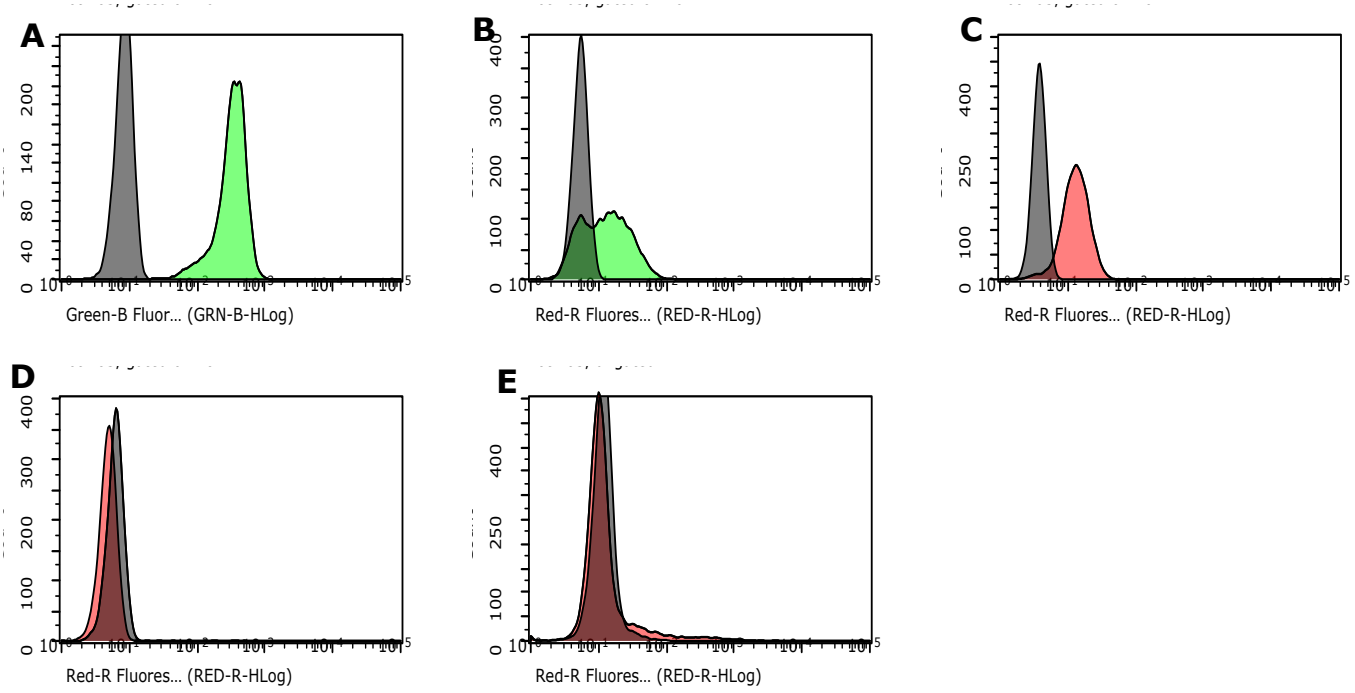

Figure 2. Cells retain characteristics of fetal-derived hematopoietic precursors with positive expression of CD44 (**A**, MABF1556), CD34 (**B**, BioLegend 128611), and C-Kit (**C**, BioLegend 105811) while display little to no expression for Thy1.2 (**D**, Invitrogen A14730), and Sca-1 (**E**, ZRB2309).

Protocols

Thawing the Cells

Do not thaw the cells until the recommended medium is on hand. SCC466 cells grow as suspension cells and thus do not require enzymatic detachment or dissociation. Passage when the cell density reaches 1 million cells/mL. Optimal plating density should be ~100,000 cells/mL. The cells should not be grown at excessively high densities.

1. SCC466 cells are thawed and expanded in HPC-7 Expansion Medium comprising of Iscove's Modified Dulbecco Media (I3390) containing 5% FBS (ES-009-B), L-Glutamine (TMS-002-C), 100 ng/mL Mouse Stem Cell Factor (S9915-50 µg), 75 uM Monothioglycerol (M6145; Dilute stock 1:10, use 6.5 µL/100 mL of total media) with optional 5-15 ng IL-6 I1395) and optional Penicillin/Streptomycin (P4333).

   Note: Addition of IL-6 may improve cell viability and proliferation. It is recommended to use when thawing and initial recovery, but continued use in culture is not recommended. Cell expression may change if used continually in culture.
2. Remove the vial of frozen cells from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of HPC-7 Expansion Medium (medium composition in Step 1) to the 15 mL conical tube.

   IMPORTANT: The expansion medium should be pre-warmed to 37 °C. Cold medium may result in decreased cell viability.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 200 x *g* for 3 minutes to pellet the cells.

   IMPORTANT: Higher spin speed and/or longer centrifugation may result in decreased viability.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative.
9. Resuspend the cells in 15 mL of HPC-7 Expansion Medium.
10. Transfer the cell mixture to a T25 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing the Cells

1. HPC-7 cells are a suspension cell line. They should be passaged before reaching 1 million cells/mL. The cells are recommended to be subcultured to a minimum cell density of 100,000 cells/mL. HPC-7 cells require media changes every 2 days. Cell viability may drop if media is not changed on a regular basis.
2. To change media, centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
3. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
4. Add desired volume of HPC-7 Expansion Medium to the conical tube and resuspend the cells thoroughly. Large cell clumps may be broken up by gentle trituration.

   IMPORTANT: Do not vortex the cells.
5. Count the number of cells using a hemocytometer or a Scepter™ 3.0 handheld automated cell counter.
6. Plate the cells to the desired density.

Cryopreservation of the Cells

SCC466 cells may be frozen in the expansion medium with 10% DMSO using a Nalgene slow freeze Mr. Frosty® container.

References

1. Pinto do O P. 1998. Expression of the LIM-homeobox gene LH2 generates immortalized Steel factor-dependent multipotent hematopoietic precursors. The EMBO Journal. 17(19):5744–5756. doi:https://doi.org/10.1093/emboj/17.19.5744.
2. Kavanagh DPJ, Yemm AI, Zhao Y, Frampton J, Kalia N. 2013. Mechanisms of Adhesion and Subsequent Actions of a Haematopoietic Stem Cell Line, HPC-7, in the Injured Murine Intestinal Microcirculation In Vivo. Thumbikat P, editor. PLoS ONE. 8(3):e59150. doi:https://doi.org/10.1371/journal.pone.0059150.
3. Kavanagh DPJ, Yemm AI, Alexander JS, Frampton J, Kalia N. 2013. Enhancing the Adhesion of Hematopoietic Precursor Cell Integrins with Hydrogen Peroxide Increases Recruitment within Murine Gut. Cell Transplantation. 22(8):1485–1499. doi:https://doi.org/10.3727/096368912x653192.

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