User Manual

HMC-1.1 Human Mast Cell Line

Cancer Cell Line

SCC067

FOR RESEARCH USE ONLY

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

Product Overview

HMC-1 cell line was derived from a patient with mast cell leukemia. These cells are widely used in studies of human mast cell function because they exhibit many key characteristics of tissue mast cells, such as expression of histamine, tryptase, heparin, and they demonstrate a similar cell surface antigen profile.[1, 2] Receptor tyrosine kinase KIT is expressed on mast cells and plays an important role in their proliferation, function, and survival. Mutations in KIT have been linked to dysregulated growth of mast cells and are associated with mast cell tumors and systemic mastocytosis. Two activating point mutations in KIT have been reported in the HMC-1 cell line.[3] KIT D816V mutation is an amino acid substitution of aspartate to valine at residue 816, located in the phosphotransferase domain of the receptor. KIT V560G mutation is an amino acid substitution of valine to glycine at residue 560 located in the juxtamembrane region between the transmembrane and tyrosine kinase part of the receptor. Both mutations convert the receptor to a constitutively tyrosine-phosphorylated and active state, leading to growth factor-independent proliferation.

HMC-1.1 (HMC-1^{560}) and HMC-1.2 (HMC-$1^{560, 816}$) are two variant sublines of the HMC-1 cell line.[2] HMC-1.1 possess the V560G mutation, but not the D816V mutation. HMC-1.2 possess both the V560G and D816V mutations and exhibits a higher proliferative rate than HMC-1.1, which has been attributed to the D816V mutation.

Short Tandem Repeat (STR) Profile

D3S1358	15	D16S539	10, 12
TH01	7, 9.3	CSF1PO	10,1 3
D21S11	29	Penta D	11
D18S51	14, 20	vWA	19
Penta E	9, 19	D8S1179	12
D5S818	12, 13	TPOX	8, 11
D13S317	11	FGA	18, 20
D7S820	10, 11	Amelogenin	X

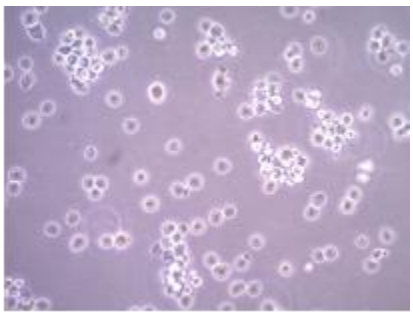
Storage and Stability

HMC-1.1 Human Mast Cell Line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages without significantly affecting the cell marker expression and functionality.

Quality Control Testing

- Each vial contains ≥1 X 10^6 viable cells.
- Cells are tested negative for HPV-16, HPV-18, Hepatitis A, B, C, and HIV-1 & 2 viruses by PCR.
- Cells are negative for mycoplasma contamination.
- Each lot of cells are genotyped by STR analysis to verify the unique identity of the cell line.

Data Analysis



Day 1

References

1. Nilsson, G, Blom, T., Kusche-Gullberg, M., Kjellen, L, Butterfield, J.H., Sundstrom, C., Nillson, K., & Hellman, L. (1994) Phenotypic Characterization of the Human Mast-Cell Line HMC-1. Scand. J. Immunol 39: 489-498.
2. Sundstrom, M., Vliagoftis, H., Karlberg, P., Butterfield, J.H., Nilsson, K., Metcalfe, D.D., & Nilsson, G., (2003) Functional and phenotypic studies of two variants of a human mast cell line with a distinct set of mutations in the c-kit proto-oncogene. Immunology 108: 89-97.
3. Furitsu, T, Tsujimura, T., Tono, T., Ikeda, H., Kitayama, H., Koshimizu, U., Sugahara, H., Butterfield, J. H., Ashman, L.K., Kanayama, Y, et al. (1993) Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. J. Clin. Invest. 92(4): 1736-44.

Protocol

HMC-1.1 are suspension cells that are very small in size. They do not grow well in roller flasks. Growth should be by static culture with a T25 flask in an upright position. Passage when the cell density reaches 1 to 1.5 million cells/mL. Optimal plating density should be ~250,000 cells/mL.

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Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue culture ware surfaces without any additional coating.

   Cells are thawed and expanded in HMC Expansion Medium containing Iscove's Modified Dulbecco's Medium (Cat. No. I6529), 1.2 mM α-thioglycerol (Cat No. M6145-100ML), 10% FBS (Cat. No. ES-009-B) and 1X Antibiotic-Antimycotic Solution (Cat. No. A5955), optional.

2. Remove the vial of frozen HMC-1.1 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 HMC Expansion Medium (Step 1 above) to the 15 mL conical tube.

   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 300 x *g* for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 10-15 mL of HMC Expansion Medium.
10. Transfer the cell suspension to a T25 flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

IMPORTANT: Set the T25 flask in an upright position in the incubator.

Subculturing Cells

HMC-1.1 suspension cells require media replenishment every 2-3 days. Passage cells when the cell density is at 1-1.5 million cells/mL.

1. Remove flask from incubator, tighten cap and place in tissue culture hood.
2. Dislodge any cells that may adhere to the flask by firmly rapping the side of the flask with the palm of the hand and gently swirl the medium over the cells to mix. Visually inspect flask to ensure the cells have been dislodged and the suspension is free of contaminants.
3. Determine cell count and viability using a hemocytometer or automated cell counter.
4. Cells are typically plated at a density of 250,000 cells/mL.

Cryopreserving Cells

HMC-1.1 cells can be frozen in the expansion media plus 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container (Cat. No. C1562).

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