Data Sheet

MNK-3 Mouse Innate Lymphoid Cell Line

Immortalized Cell Line

SCC628

Pack Size: ≥ 1 x 10^6 viable cells/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

Innate Lymphoid Cells (ILCs) contribute to the innate immune system through cell signaling and regulation of immune cells. ILCs lack functionally rearranged T-Cell Receptors (TCRs) or immunoglobulin genes.1 ILCs have been categorized into three subsets. Group 1 ILCs include Natural Killer cells (NK) and other cells that produce IFN-γ but are distinct from NK cells.[1] Group 2 ILCs are classified by their capability to produce cytokines, especially IL-5 and IL-13. Group 2 ILCs are sometimes known by variable names such as innate helper type-2 cells or natural helper cells.[1] Group 3 ILCs are classified by the expression of Rorγt which is also needed for their development. This classification includes Lymphoid Tissue inducer cells (LTi) in addition to adult Rorγt+ ILC cells.

ILC3 cells are important for lymphoid tissue development, mucosal homeostasis, containment of commensal bacteria, inhibition of the pathology that causes inflammatory bowel disease, thymic regeneration, control of mucosal IgA production, and immune defense capabilities against bacterial and fungal infections. ILC3 cells are also an important source of IL-22 which aids in the protection of intestinal mucosa from pathogens.[1]

MNK-3 is a novel, prototypical ILC3 cell line model which expresses the key transcription factors, cytokine secretion, cytokine receptors, chemokine receptors, and adhesion molecules represented in ILC3 cells. The MNK parental cell line was generated from sorted NIH-Swiss mouse fetal day-15 NK1.1+ thymocytes which were later transfected with constructs encoding the SV40 large T antigen and human c-MYC. Parental MNK cells were sorted to be NK-receptor negative. Two sublines were developed from parental MNK and later designated MNK-1 and MNK-3. NKR- MNK parental cells were cultured in IL-7 and IL-15 and confirmed to be negative for CD94, NKG2D, DX5, and CD11b. These NKR-cells became the MNK-3 subline.[1]

MNK-3 cells display LTi-like activity in vitro when incubated with mesenchymal stromal cells. A low ICAM-1, high VCAM-1 OP9 variant cell line was co-cultured with MNK-3 cells which resulted in a significant increase in ICAM-1 expression. This is essentially used as a readout for LTi activity.[1] MNK-3 cells were confirmed by ELISA to secrete IL-22 at a basal level in addition to increased IL-22 secretion when stimulated by IL-1β, IL-23, and IL-2. MNK-3 cells also serve as a model for a high number of applications presented by ILC3 functions such as IL-17A induction. MNK-3 cells are also easy to genetically modify which can aid in application function.

Source

GMO

Cell line generated from sorted NIH-Swiss mouse NK1.1+ thymocytes. Cells were infected with a Bcl-2 retroviral producer cell line and transfected with replication-deficient SV40 and human c-myc constructs by electroporation.

Short Tandem Repeat

M18-3: 18	M4-2: 18.3	M6-7: 12	M19-2: 11	M1-2: 17	M1-1: 15	M7-1: 26, 30
M3-2: 14	M8-1: 15	M2-1: 9	M11-2: 16	M17-2: 13	M12-1: 20	M5-5: 14, 15
MX-1: 26	M13-1: 16.2	M15-3: 20.3, 22.3, 23.3	M6-4: 15.3, 16.3			

Quality Control Testing

- The MNK-3 Innate Lymphoid cells are verified to be of mouse origin and negative for human, rat, Chinese hamster, Golden Syrian hamster, and nonhuman 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

The MNK-3 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

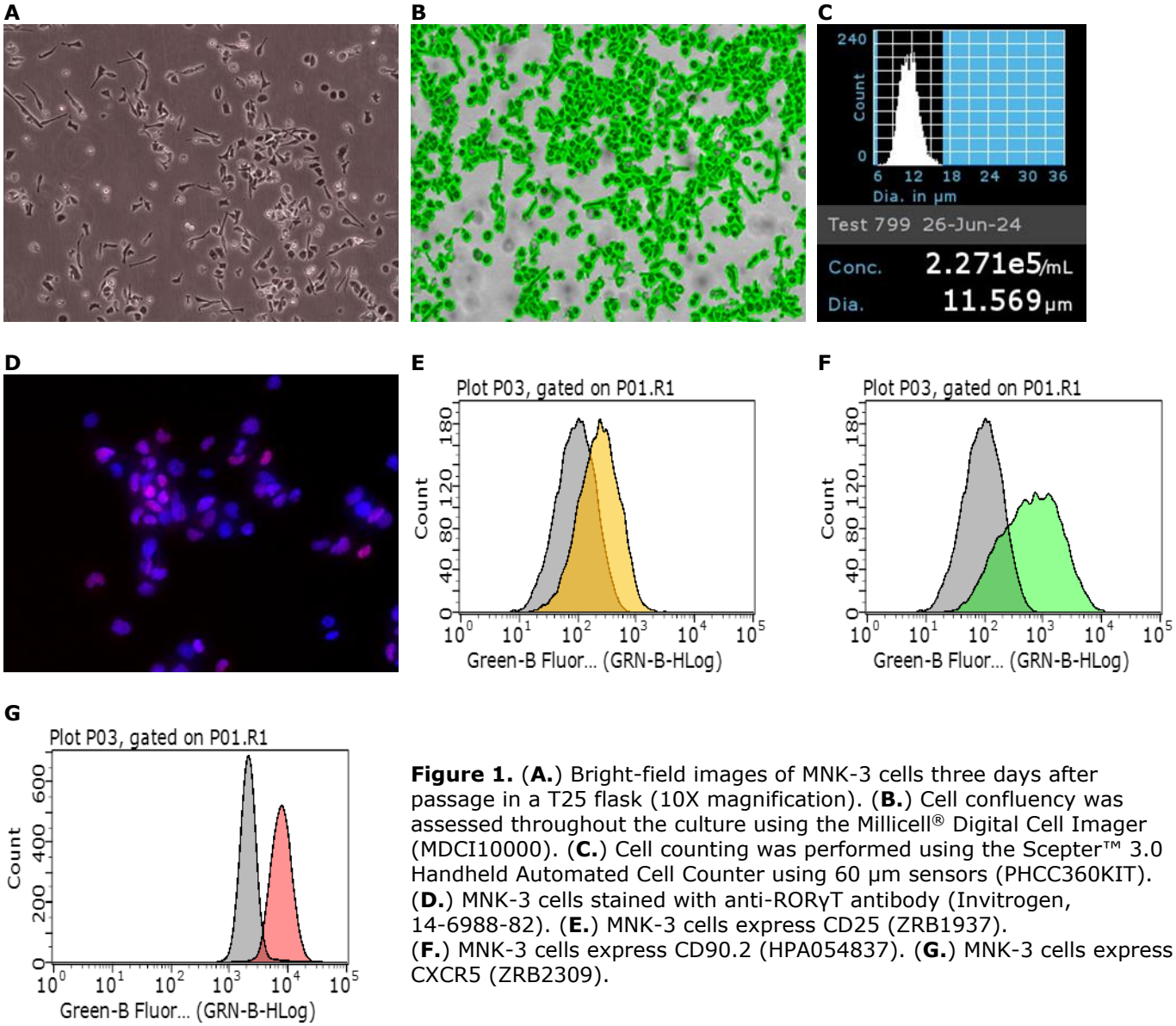

Figure 1. (**A.**) Bright-field images of MNK-3 cells three days after passage in a T25 flask (10X magnification). (**B.**) Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (MDCI10000). (**C.**) Cell counting was performed using the Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensors (PHCC360KIT). (**D.**) MNK-3 cells stained with anti-RORγT antibody (Invitrogen, 14-6988-82). (**E.**) MNK-3 cells express CD25 (ZRB1937). (**F.**) MNK-3 cells express CD90.2 (HPA054837). (**G.**) MNK-3 cells express CXCR5 (ZRB2309).

Protocols

Thawing the Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on standard tissue cultureware surfaces without any additional coating. MNK-3 cells are thawed and expanded in MNK-3 Expansion Medium comprising of DMEM (D5796) containing 10% Heat Inactivated FBS (ES-009-B), 2 mM L-Glutamine (G7513), 1X Sodium Pyruvate (TMS-005-C), 1X 2-Mercaptoethanol (ES-007-E), 1X HEPES (TMS-003-C), 10 ng/mL Mouse IL-7 (I4892), 10 ng/mL Mouse IL-5 (I1145), and Penicillin/Streptomycin (P4333) (optional).
2. Remove the vial of frozen MNK-3 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 MNK-3 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 mL of MNK-3 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.

    Note: Cells may struggle upon initial thaw. It is important to allow cells a modest amount of time to initially adhere to flask and grow during their first passage. If cells form small, tight colonies, it may be necessary to detach the cells and re-seed them to distribute cells throughout the flask and allow proper space between cell groupings.

Subculturing the Cells

1. MNK-3 cells can be passaged at ~40-60% confluency.

   Note: Cells should not grow past 60% confluency, or cells may change morphology and differentiate.
2. Carefully remove the medium from the tissue culture flask containing the 40-60% confluent layer of MNK-3 cells.
3. Rinse the flask with 10 mL 1X sterile PBS (TMS-012-A). Aspirate after the rinse.
4. Apply 5-7 mL of pre-warmed Accutase® (A6964) and incubate in a 37 °C incubator for 5 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5-7 mL of MNK-3 Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of MNK-3 cell 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.
11. Count the number of cells using a hemocytometer or a Scepter™ 3.0 Handheld Automated Cell Counter.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

References

1. Allan DS, Kirkham CL, Aguilar OA, Qu LC, Chen P, Fine JH, Serra P, Awong G, Gommerman JL, Zúñiga-Pflücker JC, et al. 2014. An in vitro model of innate lymphoid cell function and differentiation. Mucosal Immunology. 8(2):340–351. doi:https://doi.org/10.1038/mi.2014.71.

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