Cell Lines Used for Research Only

NK-92MI

Cat. No. : CL-0533

Origin and General Characteristics

Cell Name	NK-92MI
Organism	Human
Age	50Y
Sex	Male
Tissue	Peripheral blood
Morphology	Lymphoblast-like
Growth Properties	Suspension
Background	The NK-92 cell line is an interleukin-2 (IL-2)-dependent natural killer (NK) cell line derived from peripheral blood mononuclear cells of a 50-year-old Caucasian male with rapidly progressive non-Hodgkin's lymphoma. The NK-92MI cell line is an IL-2-independent NK subline generated from NK-92 cells via retroviral transduction. Parental NK-92 cells were transduced with human IL-2 cDNA carried by the retroviral MFG-hIL-2 vector; transduction was stable, presumably due to integration of the vector into the host genome. NK-92 cells are cytotoxic to a wide range of malignant cells; chromium-release assays confirm their ability to kill K562 and Daudi cells. NK-92 cells exhibit the following immunophenotypic characteristics: positive for surface markers CD2, CD7, CD11a, CD28, CD45, and CD54; negative for CD1, CD3, CD4, CD5, CD8, CD10, CD14, CD16, CD19, CD20, CD23, CD34, and HLA-DR. The parental IL-2-dependent NK-92 cell line and a second IL-2-independent subline, NK-92CI, are available from the ATCC. Both NK-92MI and NK-92CI variants contain and express the hIL-2 cDNA and secrete bioactive IL-2; NK-92MI cells produce higher levels of IL-2 than NK-92CI cells, while parental NK-92 cells do not produce IL-2. A culture submitted to ATCC in September 1998 was found to be contaminated with mycoplasma; the progeny were cured of mycoplasma via 21-day treatment with BM Cyclin. Six weeks post-treatment, mycoplasma testing via Hoechst staining, PCR, and standard culture all yielded negative results.
Biosafety Level	BSL-2

Culture Conditions and Handling

Complete Medium	MEMα[PM150422]+0.2 mM Inositol+0.1 mM Supplement1+0.02 mM Folic Acid+12.5% Nutrient2+12.5% Nutrient1+1% Supplement2
Subcultivation Ratio	5×10^5-1×10^6 cells/mL
Medium Renewal	2 to 3 times per week
Incubation Atmosphere	Air, 95%; CO_2, 5%
Temperature	37°C
Freeze medium	Freezing Medium (Serum-free & animal origin-free)[PB180438]

Storage Conditions	For long-term cryopreservation, cryovials should be stored in liquid nitrogen at −150°C to −196°C. Storage at −80°C is restricted to short-term interim use only.
Instructions	1. Check all containers for leakage or breakage. 2. Remove the frozen cells from the dry ice packaging and immediately transfer them to liquid nitrogen (liquid or vapor phase) for long-term cryopreservation. 3. This cell line is a suspension cell line. Harvest the cell suspension by centrifugation; do not discard the culture medium directly.
Subculturing Procedure	Cultures can be maintained by either supplementing with fresh medium or exchanging medium via centrifugation. The recommended centrifugation conditions are 1200 rpm (approximately 250 × g) for 3 minutes.

Handling Precautions for NK-92MI Cells

1. NK-92MI cells grow in suspension and predominantly form aggregates, with only a small proportion of single cells. Abundant dead cells and cellular debris are often visible between cell clusters.
2. Maintain adequate IL-2 stock in the lab. If cells display poor viability, a higher proportion of scattered single cells or growth arrest, supplement the medium with IL-2 to a final concentration of 200 U/mL. Cellular morphology and proliferative capacity recover after 2–3 days of incubation.
3. NK-92MI cells are sensitive to centrifugal force. Conduct medium replacement every 2–3 days by alternating half-medium replenishment and full medium exchange via centrifugation: perform 2–3 rounds of half-medium replenishment, then carry out one full medium change. Minimize centrifugation steps; avoid centrifugation when cell viability or cell density is low.
4. Medium Exchange Protocols

   Medium replenishment method: Add an appropriate volume of fresh medium every 2–3 days (2 mL per T25 flask). Perform one full medium change by centrifugation after 2–3 replenishment cycles.

   Half-medium exchange method: Gently tap the flask to dislodge loosely adhered cell aggregates, then stand the flask upright to let cells settle. Carefully aspirate 2.5 mL supernatant and transfer it into a centrifuge tube. Centrifuge at 1200 rpm (approximately 250 × g) for 3–5 min and inspect for cell pellets to prevent cell loss. Add 2.5 mL fresh medium back to the original flask. If cells are in the expansion phase with low density, resuspend the pelleted cells in fresh medium and transfer the suspension back to the original flask. Cell aggregates gradually enlarge during cultivation. Healthy cell clusters appear phase-bright under a microscope. Subculture is required when clusters become excessively compact with darkened central regions.
5. **Subculturing Procedure**

   Gently dissociate cell aggregates by pipetting up and down 2–3 times. Vigorous pipetting is prohibited, as it triggers massive cell death and debris generation. Split the uniform cell suspension equally into two flasks, then supplement each flask with an equal volume of fresh medium.

NK-92MI cells have high nutritional demands. Excessively large aggregates lead to rapid nutrient depletion. Gently disrupt oversized clusters with limited pipetting; full dissociation into single cells is unnecessary.

Notes on Dissociation of Cell Aggregates

1. Minimize centrifugation times and control pipetting cycles during subculture. Complete dissociation into single cells is not required, although gentle dissociation is allowed.
2. When performing routine subculture or medium exchange after centrifugation, regulate both the frequency and force of pipetting. Even if cells are fully separated into single cells, they will re-aggregate within 1–2 days.
3. Partially dissociate excessively large cell clusters.
4. Fully disperse cells before cryopreservation.

Handling Recommendations for Cryopreserved Cells

Preparations Before Thawing

1. Prepare the recommended complete culture medium and preheat it to 37°C for 30 minutes.
2. Prepare 9 mL of complete medium within a sterile centrifuge tube to dilute the cryoprotectant present in the frozen cell suspension.
3. Use sterile gloves to protect the cryovial from contact with the water bath water, and preventing cell contamination.

Thawing Procedure

1. Retrieve the cryovial containing frozen cells from liquid nitrogen storage. Place it in sterile gloves and immediately submerge it in a 37°C water bath.
2. Thaw the cells rapidly (<1 minute) by gently swirling the vial in the 37°C water bath until only a small ice crystal remains.
3. Transfer the vial to a biosafety cabinet. Wipe the exterior of the vial with 75% ethanol before opening.
4. Transfer the thawed cells dropwise into a preheated 9 mL centrifuge tube containing complete medium.
5. Centrifuge the cell suspension at approximately 250 × g for 5 minutes.
6. After centrifugation, discard the supernatant and retain the cell pellet.
7. Gently resuspend the cells in complete growth medium, then transfer them to an appropriate culture vessel and place it in the recommended culture environment.

Notes

1. The entire recovery process should be completed as quickly as possible.
2. Select the culture vessel size based on the number of cells in the cryovial. Procell single cryovials are recommended for resuspension in T25 flasks or 10 cm dishes.
3. Minimize the time thawed cells are kept at room temperature. Cryoprotectant must be immediately diuted or removed by centrifugation.
4. Place the flask upright in the incubator with the cap facing upward to increase local cell density; ensure the cap remains vented for gas exchange.
5. NK-2 cells require a stable culture environment. Perform microscopic observation once daily and

avoid frequent removal of flasks from the incubator.

6. Post-thaw, replenish with fresh medium every 3 days. Add 1–2 mL fresh medium each time, followed by a half-medium exchange after 1–2 supplementation rounds. Minimize centrifugation steps.
7. Subculture when the medium turns visibly yellow and cell aggregates expand prominently. Do not splt the culture into more than one flask of matching size per passage.

Precautions After Receipt of Frozen Cells

1. Upon unpacking, inspect the condition of the frozen cells and dry ice, and take photographs immediately. The following after-sales service will be provided based on these photographs, including assessment of the remaining dry ice, verification that the cryovial was fully buried in dry ice, and evaluation of whether the cells thawed and refrozen during transit.
2. Upon receipt, the cells should be transferred to liquid nitrogen immediately or directly resuscitated. If liquid nitrogen is unavailable, the cells can be temporarily stored at -80°C, however, the storage period should be limited to less than two weeks whenever possible. Prolonged storage at -80°C may gradually reduce post-thaw cell viability, and the extent of this viability loss is unpredictable.
3. Ensure that the operator has sufficient knowledge and experience in cell culture, and the laboratory is equipped with essential instruments, including a biosafety cabinet, CO_2 incubator, inverted microscope, centrifuge, water bath. Carefully review the cell instruction manual to understand key cell characteristics including growth properties (adherent or suspension), morphology, basal medium requirements, serum concentration, cytokine supplementation, subcultivation ratio, medium renewal schedule.
4. After resuscitation, observe the cells under a microscope and record the cell status by taking photographs (1-3 images each at 100× and 200× magnification for 3 consecutive days). These images will serve as supporting documentation for follow-up services. In addition, a small aliquot of cells may be used to assess cell viability by automated cell counting or trypan blue staining.
   Note: Cells should not be observed too frequently within the first 24 hours after resuscitation, as this may affect cell growth or adherence. Observation once per day is sufficient.
5. After sufficient expansion, the cells should be cryopreserved as soon as possible to ensure availability of backup stocks for future experiments or in case of unexpected experimental failure. Cells in the logarithmic growth phase should be selected for cryopreservation. After cryopreservation, it is recommended to resuscitate one vial to verify post-thaw viability and confirm successful cryopreservation.