Gentaur

Product Information Sheet

Cell Line Designation: NK-92 MI

Catalog No. C0003051

Cell Line Description:

Disease: Malignant non-Hodgkin's lymphoma

Origin: NK-92 MI is an interleukin-2 (IL-2) dependent natural killer cell line derived from NK-92 cell line by transfection. NK-92 is an interleukin-2 (IL-2) dependent Natural killer cell line derived from peripheral blood mononuclear cells from a 50-year old Caucasian male with rapidly progressive non-Hodgkin's lymphoma. The parental cells were transfected with human IL-2 cDNA in the retroviaral MFG-hIL=2 vector by particle-mediated gene transfer. The transfection is stable.

Species: Homo sapiens, human

Tissue: Peripheral blood

Properties: Lymphoblast, suspension, multicell aggregates

Complete Medium: Supplemented Alpha Minimum Essential medium (Supplemented αMEM) (C0013-51) (adapted to NK-92 and NK-92MI cells) and add the following components to the base medium:

Adjust to a final concentration of 12.5% horse serum (C0013-52) and 12.5% FBS (C0013-12)

Subculture Procedure: Cultures can be maintained by addition or replacement of medium. When replacing media, centrifuge cells and resuspend cell pellet in fresh medium at 2-3x10^5 viable cells/mL. Centrifugation and full replacement of culture medium may be performed for the first subcultures. Cultures can then be maintained by addition of fresh medium. These cells tend to gro in aggregates that may lose viability when they are dispersed. Accurate counts and viabilities may not be possible. Culture at 5% CO_2, 37°C. Maintain cell density between 2x10^5 and 10^6 viable cells/mL or use a 1:3 split ratio.

Medium Renewal: Replace with fresh medium every 2 to 3 days (depending on cell density).

Freezing Medium: 90% FBS; supplemented with 10% (v/v) DMSO

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. laboratory safety is discussed in the following publication: Biosafety in Microbiological and Biomedical Laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is also available online at www.cdc.gov/od/ohs/biosafty/bmbl4/bmbl4toc.htm

Use Restrictions: **These cells are distributed for research purposes only.** Gentaur does not recommend third party distribution of this cell line, as this practice has resulted in the unintentional spreading of contaminated cell lines.

Handling Procedure for Frozen Cells:

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

Safety Precaution:

Gentaur highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.

3. Transfer the vial contents to the centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125xg for 5 to 7 minutes.

4. Resuspend cell pellet with the recommended complete medium and dispense into a new culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6).

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended.

References for NK-92 MI cells:

1. Gong JH, Maki G, Klingemann HG. Characterization of a human cell line (NK-92) with phenotypical and functional characteristics of activated natural killer cells. Leukemia. 1994;8(4):652-658.

Lot Specific Information Sheet for Cat #: C0003051

Lot Number: 0002236

Designation: NK-92 MI CELLS

Total Cells/mL: >1.0x10^6

Expected Viability: 56.8%

Ampule Passage #: 26

Dilute Ampule Content: 1:10 (T-25) or 1:15 (T-75)

Volume/Ampule: 1 mL

A T-25 setup at a dilution of 1:10, using culture medium as described in the product information sheet, at a seeding density of 3.0x10^5 viable cells/mL reaches approximately 3.4x10^5 viable cells/mL in 2 days.

Remarks:

Please clearly read this instruction manual and compare the medium formulation before proceeding.

NK-92MI cells have proven difficult to recover from cryopreservation, presenting with heavy cell debris, low post-freeze viabilities and poor proliferation. This cell line displays an extended lag phase during recovery from cryopreservation. It can take as long as 14 days before the cells grow out to a density acceptable for expansion. It is normal to observe heavy cell debris upon thaw. It is also significant to note that even when the cells reach the log phase of growth, NK-92MI can still be difficult to maintain in culture.

NK-92MI is sensitive to overcrowding and requires strict maintenance between a density of about 2 x 10^5 and 1 x 10^6 cells/mL (slightly higher cell densities are used during post-freeze recovery). The cells grow as aggregates that tend to lose viability when broken apart into single cells. However, to maintain density requirements and selective pressure on the cells, cultures should have their fluid changed about every 2-3 days. This involves the use of mechanical manipulation to break up large clumps resulting in a decline in viability just after the media is changed or the cells are subcultured. We recommend removing the cells and transfer the cell pellet using a pipet slowly to avoid excessive break-up of the cell pellets. Centrifugation and full replacement of medium may be performed for the first subculture. Cultures can then be maintained by addition of fresh medium. Pipet the cells up and down on the back of the flask every 2-3 days to produce a single cell suspension. Maintain cell density between 2 x 10^5 and 1 x 10^6 viable cells/mL or use a 1:3 split ratio. For additional information pertaining to the development, characteristics and growth of NK-92MI cells, please refer to the following reference:

Tam YK, Maki G, Miyagawa B, Hennemann B, TonnT & Klingemann HG. *Characterization of genetically altered, interleukin 2-independent natural killer cell lines suitable for adoptive cellular immunotherapy*. Hum Gene Ther. 10:1359-1373, 1999.

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