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We use this protocol and it's working

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NK-92 Cell Culture Protocol

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ABSTRACT

NK-92 cells are an interleukin-2 (IL-2)-dependent natural killer (NK) cell line derived from peripheral blood mononuclear cells of a 50-year-old white male with acute non-Hodgkin lymphoma.

GUIDELINES

- NK-92 cells are an interleukin-2 (IL-2)-dependent natural killer (NK) cell line derived from peripheral blood mononuclear cells of a 50-year-old white male with acute non-Hodgkin lymphoma.
- The cells are rounded or elliptic, transparent and grow in clumps.
- Only clusters of cells are proliferative, and individual cells are essentially no proliferative. Clumps of cells may grow into large, visible clumps, and dead cells may shrink, lose their brightness, and become gray.
- NK-92 cells are very sensitive to oxygen, nutrients and cell density, and apoptosis is easy to happen when nutrients are insufficient or density is too high.

METHODS

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Mima basal medium	370 mL

Fetal bovine serum	62.5 mL
HS (Horse Serum)	62.5 mL
P/S penicillin-streptomycin	5 mL
Inositol	0.2 mM (500 µL)
β-mercaptoethanol	0.1 Mm (1 mL)
Folic acid (folic acid)	0.02 mM (180 µL)
Recombinant IL-2 interleukin	100-200 U/mL (100 µL)

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- An incomplete medium is prepared in advance. αMEM+0.1 mM β-mercaptoethanol + HS and FBS at a final concentration of 12.5%. Interleukin-2 (IL-2) is added to the medium when used (Table 1).
- When cells are just recovering or at low density, the culture flask can be kept upright to increase cell density and promote cell agglomeration. When the cell mass is growing normally, it is recommended that the culture flask be laid flat to increase the area of air exchange, and the liquid height should not exceed 3 mm. Generally, 6-8 mL of culture medium is added to the T25 culture flask, and about 15 mL of culture medium is added to the T75 culture flask.
- Fluid change. Change culture medium every 2-3 days. Semi-liquid exchange method. During exchanging culture medium, the culture bottle/dish is turned on its side. Avoid shaking the cells. Part of the supernatant is gently absorbed, discarded with a straw, and then add the same volume of fresh culture medium to continue the culture. For centrifugation, all in the culture bottle are transferred to centrifuge tubes. Centrifuge at 1000 rpm for 5 minutes, and remove the supernatant. The sediment left behind is gently suspended, and then re-seeded in flasks. Do not blow too much during resuspension.
- Passage. Passage should be performed when there are 4-5 large cell clusters at 20× field. A cell mass of 2 times the volume of fresh culture medium is added during passage. Then blow gently to break the large cell mass into small cell mass, but do not blow hard into single cell suspension, which is basically free of proliferating cells. And transfer the mixed cell suspension to a new culture flask. Alternatively, the cell suspension is transferred to a centrifuge tube, centrifuged at 1000 rpm for 5 minutes. Then the supernatant is discarded. Add a triploid volume of fresh culture medium, gently re-suspend and transfer it to three new culture flasks.
- Cell cryopreservation. After cell counting, $1\times10^6$to 1×10^7cells from NK-92 are collected and centrifuged at 750 rpm for 5 minutes. The original culture medium is discarded, and 1 mL of cryopreservation solution is added. The mixture is labeled and sealed, and stored in a frozen storage box at -80°C for 24 hours. Put it in liquid nitrogen.

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- IL-2 can be used separately, but avoid repeated freeze-thaw.
- When transferring cells, cells should not be placed outside the incubator for too long.
- Resuspension of NK-92 cells should be gentle, not too violent.
- It is best not to add antibiotics, if you must add, add 1% green streptomycin.
- The whole fluid is better than the half fluid, which is passed every 48 hours.
- IL-2 must be 100-200 U/mL for cell culture, and the concentration of IL-2 does not need to be increased any more. If the concentration is too high, the cell growth will be affected, and the

concentration cannot be reduced any more.

- Cell clumps need to be blown away, but do not deliberately blow into a single piece.

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