ExCell

EIV12026E

ExCell Bio

NK Serum-free Medium NH04

For Research and Manufacturing Use

Not Intended for Diagnostic and Therapeutic Use

User Manual

Catalog Number	NH000-N042



PRODUCT DESCRIPTION

NK Serum-free Medium NH04 is a comprehensive, serum-free, xeno-free kit designed for the activation and expansion of NK cells. This kit encompasses everything needed for the robust expansion of NK cells, including:

NK Serum-free Activation Medium 2.0: This medium is used for the initial 7-day culture of NK cells, providing a foundational environment for their activation.

NK Serum-free Basal Medium 2.0: This medium serves as the base for subsequent expansion of NK cells beyond the initial activation phase.

OptiVitro® Immune Cell Serum-free Medium Supplement UE01: It is added to the Activation/Basal Medium to create a complete medium that supports the activation and expansion of NK cells.

NK Coating Reagent, NK Activation Reagent, and NK Expansion Reagent: Crucial components for supporting NK cell activation and expansion.

This kit is meticulously formulated to support the selective expansion of NK cells derived from human peripheral blood mononuclear cells (PBMCs) and umbilical cord blood mononuclear cells (CBMCs).

SPECIFICATION, STORAGE AND TRANSPORTATION REQUIREMENT

Product Name	Cat. #	Specification	Quantity	Storage	Transportation	Shelf Life
NK Serum-free Medium NH04	**NH000-N042**	**2.2 L kit**	-	-	-	-
NK Serum-free Activation Medium 2.0	BA0431	200 mL	1	2-8°C Protect From Light.	< 25°C Protect From Light.	12 months
NK Serum-free Basal Medium 2.0	BA0442	1000 mL	2	2-8°C Protect From Light.	< 25°C Protect From Light.	12 months
OptiVitro® Immune Cell Serum-free Medium Supplement UE01	BA0332	8 mL	2	2-8°C Protect From Light.	< 25°C Protect From Light.	18 months
OptiVitro® Immune Cell Serum-free Medium Supplement UE01	BA0331	4 mL	1	2-8°C Protect From Light.	< 25°C Protect From Light.	18 months
NK Coating Reagent	BA0452	100 µL	1	-20°C * Protect From Light.	< 0°C Protect From Light.	12 months
NK Activation Reagent	BA0462	200 µL	1	-20°C * Protect From Light.	< 0°C Protect From Light.	12 months

NK Expansion Reagent	BA0472	500 µL	2	-20°C * Protect From Light.	< 0°C Protect From Light.	12 months

***Note:** It is suggested to store the reagents at -80*°C for long-term storage.

HANDLING RECOMMENDATIONS

1. Please make sure to store the cell culture medium in a light-protected environment, avoid fluorescent lamps or other lamplight exposure, and better to use colored packaging bags in the refrigerator or warehouse.
2. During the transportation of the product, it should be kept away from light. This is to prevent the product from being affected by the irradiation of fluorescent lamps or other light sources, which may lead to discoloration.
3. During the transportation of the product to the clean area, it is essential to carry out a cleaning process. The cleaning method may involve disinfectant wiping, and not utilize UV irradiation.

   ***Note:** When passing through transfer windows equipped with UV lamp, remember to proactively turn off the UV lamp inside the transfer window.*
4. The medium can be used with heat-inactivated autologous plasma, commercial human platelet lysate products, or human AB serum, but not with ICSR products.
5. Upon receipt, check for dry ice in the package and ensure the cytokines are frozen. If normal, immediately store at below -20°C, protect from light. For any abnormality, contact the seller as soon as possible.

INSTRUCTION FOR USE

Separation of human peripheral blood mononuclear cells

1. Reagent preparation

   Collect around 50 mL blood into sodium heparin blood collection tubes to prevent clotting. Equilibrate peripheral blood, DPBS (normal saline), and lymphocyte separation solution (recommended GE brand, Cat. # 17144002) to room temperature before proceeding with mononuclear cell isolation. It is recommended to perform cell separation as soon as possible after blood collection, ideally within 8 hours, to ensure cell viability and integrity.
2. Plasma extraction

   Distribute the peripheral blood evenly into 50 mL centrifuge tubes, 15-20 mL each, and centrifuge at 800×g for 10 min at room temperature with a gradual acceleration (speed up to 8) and deceleration (speed down to 3). Carefully transfer the upper layer of light-yellow plasma into new 50 mL centrifuge tubes, reserving the lower layer of red liquid for mononuclear cell extraction. Inactivate the plasma in a water bath at 56°C for 30 min, and cool down the plasma at -20°C for 15 min. Centrifuge the inactivated plasma at 1000×g for 20 min, collect the supernatant, and store it at 2-8°C.

   Note:

   1) Ensure that the plasma is clear after inactivation, as turbid plasma can affect NK cell activation.

2) *When collecting umbilical cord blood using 200 mL blood collection bag, consider the dilution effect of the anticoagulant on the plasma, it is recommended that the ratio of anticoagulant is less than 25%. When the blood volume is less than 100 mL, it is recommended to remove at least half of the anticoagulant from the blood collection bag to minimize its impact on the plasma, if the anticoagulant cannot be removed, do not use the plasma for NK cell culture, use serum replacement instead.*

3. Isolation of human peripheral blood mononuclear cells

   For peripheral blood, dilute the lower red blood cell sediment with DPBS or saline in a 1:1 ratio. For umbilical cord blood, dilute with DPBS or saline in a 1:2 ratio.

   In two new 50 mL centrifuge tubes, slowly layer the diluted blood over the lymphocyte separation solution in a 1:1 ratio (20 mL lymphocyte separation solution: 20 mL diluted blood), ensuring a distinct layer formation without mixing the diluted blood into the lymphocyte separation solution. Centrifuge at room temperature at 1200×g for 25 minutes with a gentle acceleration (speed up to 1) and deceleration (speed down to 0).

   Gently aspirate the mononuclear cells (buffy coat) and transfer to a new 50 mL centrifuge tube. Add an equal volume of DPBS and centrifuge at 400×g for 10 minutes (speed up to 9, speed down to 9) at room temperature. Discard the supernatant, wash the cells again with 40 mL DPBS, centrifuge at 400×g for 10 minutes (speed up to 9, speed down to 9), and discard the supernatant. Resuspend the cells with room temperature NK Activation Complete Medium and take a small number of cells for cell counting.

Note: *For cord blood, it is recommended to count cell number with AOPI reagent or use red blood cell lysis buffer to lyse red blood cells before counting and seeding to ensure accurate cell quantification and viability assessment.*

Medium preparation

1. NK Activation Complete Medium:

   Allow the NK Serum-free Activation Medium 2.0 and OptiVitro® Immune Cell Serum-free Medium Supplement UE01 (BA0331) to equilibrate to room temperature for 1-2 hours. Allow the NK Activation Reagent to thaw at room temperature for about 10 minutes, briefly centrifuge the vial before use. For every 200 mL of NK Serum-free Activation Medium 2.0, add 1.6 mL of OptiVitro® Immune Cell Serum-free Medium Supplement UE01 (discard the remaining 2.4 mL of Supplement UE01) and 200 µL of NK Activation Reagent. Gently mix by inverting the bottle 3-5 times to create the NK Activation Complete Medium. After mixed, the medium can be stored at 2-8°C, protect from light, and should be used within one week.

2. NK Expansion Complete Medium:

   Allow the NK Serum-free Basal Medium 2.0 and OptiVitro® Immune Cell Serum-free Medium Supplement UE01 (BA0332) to equilibrate to room temperature for 1-2 hours. Allow the NK Expansion Reagent to thaw at room temperature for about 10 minutes, briefly centrifuge the vial before use. For every 1000 mL of NK Serum-free Basal Medium 2.0, add 8 mL of OptiVitro® Immune Cell Serum-free Medium Supplement UE01 and 500 µL of NK Expansion Reagent. Gently mix by inverting the bottle 3-5 times to create the NK Expansion Complete Medium. After mixed, the medium can be stored at 2-8°C, protect from light, and should be used within one week.

Note:

1) *Allow the basal medium and supplement to equilibrate to room temperature separately before mixing, and avoid heating multiple times.*
2) *Use NK Activation Complete Medium for initial seeding on day 0 and for feeding on Day 3 (or Day 4) and Day 5 (or Day 6), switch to NK Expansion Complete Medium from day 7 onwards. Discard the extra NK Activation Medium.*
3) *Allow cytokines to thaw at room temperature for about 10 minutes, briefly centrifuge the vial before use. Repeated freeze-thaw of cytokines should not exceed 3 cycles.*

Procedure of use

1. Day 0

   Pretreatment of T75 culture flask:

   Allow NK Coating Reagent to reach room temperature. In a 15 mL centrifuge tube, add 10 mL of DPBS (saline is not recommended) and then pipette 100 μL of NK Coating Reagent into the DPBS. Mix the solution by inverting the tube gently. Add the mixed solution to a T75 cell culture flask with a bottom area of 75 cm^2, gently shake the flask back and forth to ensure even distribution of the liquid across the bottom. Coat the flask overnight (16-24 hours) at 4°C or for 2-3 hours at 37°C if immediate coating is required.

   PBMCs seeding:

   Carefully discard the coating solution without rinsing the flask with PBS to preserve the cytokine layer. Add 18 mL of NK Activation Complete Medium (pre-added with NK Activation Reagent), 10% autologous plasma (2 mL), and the PBMCs to the T75 flask. The total volume is 20 mL. Gently shake the flask back and forth to distribute the cells evenly and place it in a 37°C, 5% CO_2 incubator.

Note:

1) *Ensure that the coating solution is discarded around 10 minutes before cell seeding.*
2) *Aim for a starting cell density of 2.0×10^6 cells/mL for PBMCs seeding, increase to 2.5×10^6 cells/mL for CBMCs seeding.*
3) *When seeding cells, pipet the medium against the side wall of the T75 flask, not the bottom, to avoid disturbing the coating.*
4) *If the cell number is suboptimal, the seeding volume can be adjusted (15-25 mL) to maintain the recommended seeding density described above.*

2. Day 3

   Slowly add 18 mL of NK Activation Complete Medium (pre-added with NK Activation Reagent) and 10% heat-inactivated autologous plasma (2 mL) to the culture flask, or add the same volume of NK Activation Complete Medium and 10% heat-inactivated autologous plasma as that on Day 0.

Note: *Due to sample variability, the decision to supplement the medium should be based on the cell growth and medium color change. If cell clusters are sparse and the medium remains reddish, supplementation may be postponed for one day.*

3. Day 5

   Sample and count cells. Adjust the cell density to $0.5\text{-}0.7\times10^6$ cells/mL with NK Activation Complete Medium (pre-added with NK Activation Reagent) and 5% heat-inactivated autologous plasma, and transfer the medium and cells from the T75 flask to a T175 flask, discard the rest NK Activation Complete Medium. If the total cell

number is high, expand the culture to a total volume of 200 mL with NK Activation Complete Medium and 5% heat-inactivated autologous plasma. Then transfer the entire cell suspension from the T75 flask into two T175 flasks.

Note: *Do not exceed 100 mL of cell suspension per T175 flask.*

4. From Day 7 Onward

   Sample and count cells every 2-3 days. Supplement the culture with fresh NK Expansion Complete Medium (pre-added with NK Expansion Reagent) and 1% heat-inactivated autologous plasma. Adjust the cell density to 0.5-0.7×10^6 cells/mL. Based on the culture volume, either scale up by transferring to a larger flask or into a cell culture bag. Two days prior to harvest, readjust the cell density to 0.8-1.0×10^6 cells/mL. If the total culture volume exceeds 2 L, the basal medium, supplements, and NK expansion factors can be procured separately for medium preparation.

Note:

1) It is recommended to transfer cultures to a cell culture bag when the volume exceeds 300 mL. When using a cell culture bag, if the volume is less than 500 mL, fold the bag in half to reduce the growth surface area. For a 1.8 L capacity bag, the maximum recommended working volume is 1.5 L; for volumes exceeding this, splitting the culture into multiple bags is advised for better results.

2) For CBMCs culture, it is recommended to maintain the addition of commercial human platelet lysate or human AB serum throughout the entire process, or supplement with commercial human platelet lysate after the heat-inactivated autologous plasma is used up. For PBMCs culture, plasma supplementation is not required after the heat-inactivated autologous plasma is used up.

5. Harvest cells

   It is recommended to harvest cells on D13-D15 for PBMCs culture, and on D17-D20 for CBMCs culture.

DISCLAIMER

1. Use the product according to the manual instructions. Deviations from these instructions are at the user's risk, and our company will not be responsible for any resulting product performance deviations.
2. This product is for scientific research and commercial production only and is not intended for clinical diagnosis or treatment. Users assume all risks for unauthorized use, and our company shall not be responsible for any consequences.