www.bioscience.lonza.com
U.S. Scientific Support: 800-521-0390
scientific.support@lonza.com
EU/ROW Scientific Support: +49-221-99199-400
scientific.support.eu@lonza.com
Document # CD-OP006 08/22

Natural Killer (NK) Cell Expansion/Activation and Cytotoxicity Assay

Instructions for use

Safety statements

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.
WARNING: LONZA PRIMARY CELLS CONTAIN HUMAN SOURCE MATERIAL; TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA-approved method for the presence of HIV-I, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV-1, hepatitis B virus, and hepatitis C virus. Testing cannot offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human-sourced products should be handled at the biological safety level 2 to minimize exposure to potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th edition. If you require further information, please contact your site safety officer or Scientific Support.

Preparation of reagents for NK Cell assay

All work should be done in a laminar flow hood. Decontaminate the external surfaces of all supplement vials and the medium bottles with ethanol or isopropanol.

1. **IL-15 Working Solution**
   Make IL-15 working solution by adding 10 µL of 100 µg/mL IL-15 working solution to 990 µL H_20 for a working concentration of 1 µg/mL IL-15 (IL-15 is used to trigger NK cell expansion).
2. **IL-21 Working Solution**
   Make IL-21 working solution by adding 10 µL of 100 µg/mL IL-21 working solution to 990 µL H_20 for a working concentration of 1 µg/mL IL-21 (IL-21 is used to boost NK cell stimulatory effect).
3. **CellTracker Green Dye Working Solution**
   NOTE: Allow dye to get to room temperature prior to reconstitution of lyophilized product.
   Reconstitute lyophilized product in 200 µL DMSO to make a stock concentration of 10mM. Make a working solution by adding 0.5 µL of the 10mM stock to 999.5 µL X-VIVO® 15 Serum-free Hematopoietic Cell Medium for a working concentration of 5 µM.

NK Cell Expansion and Activation Protocol

NOTE: All work is to be performed in a laminar flow hood.

1. Pipette 10 mL of pre-warmed X-VIVO® 15 Serum-free Hematopoietic Cell Medium into 15 mL conical tube and place in laminar flow hood.
2. Thaw NK cells at 37ºC for ~2 min until only a sliver of ice remains and pipette cells into X-VIVO® 15 Serum-free Hematopoietic Cell Medium in 15 mL conical from step 1.
3. Wash cell vial with 1 mL X-VIVO® 15 Serum-free Hematopoietic Cell Medium from conical and add back to conical.
4. Centrifuge cells at 300xg for 10 minutes at 4ºC
5. Resuspend pellet in 1 mL X-VIVO® 15 Serum-free Hematopoietic Cell Medium and count using Trypan Blue and a hemocytometer at a 1:50 dilution.
6. After counting, resuspend cells at 2 x 10^6 cells/mL in X-VIVO® 15 Serum-free Hematopoietic Cell Medium and plate cells with IL-15
   a. In 24-well plate: Add 1 mL of cell suspension to each well, then add 10 µL of IL-15 working solution
   b. In 48-well plate: Add 500 µL of cell suspension to each well, then add 5 µL of IL-15 working solution
7. Incubate cells at 37ºC, 5% CO_2 for 10 days with media change every 3-4 days. Follow the following sub-steps to change media
   a. Collect all cells and supernatant into a 15 or 50 mL conical tube.
   b. Centrifuge the cells at 300xg for 10 minutes at room temperature
   c. After centrifugation, aspirate the supernatant and resuspend cell pellet in X-VIVO® 15 Serum-free Hematopoietic Cell Medium at 1 mL/well or 500 µL/well

for 24-well and 48-well culture plate, respectively.
   d. Add cells back to the culture plate at 1 mL/well or 500 µL/well for 24-well and 48-well culture plate, respectively.
   e. Add 10 µL/well or 5 µL/well of IL-15 working solution for 24-well and 48-well culture plate, respectively.
   f. Incubate cells at 37ºC, 5% CO_2
8. On day 10, follow through step 7 for media change, then add 25 µL/well or 12.5 µL/well of IL-21 working solution for 24-well and 48-well culture plate, respectively.
9. Incubate cells until day 14, then harvest cells and proceed with downstream assay(s) of interest.

NOTE: Immuno-phenotyping can be performed via FACS on Day 0 and 14 targeting CD56, CD16, CD3, NKG2D, NKp46 and NKp44 cell surface markers to assess activation status of NK cells.

NK Cell Cytotoxicity Assay

Note: The NK cells used in this experiment need to be matured and activated in culture for 14 days by following the NK Cell Expansion and Activation protocols prior to initiating the NK Cell Cytotoxicity Assay.

NOTE: K562 cells will be required for this assay. It is recommended to plate and culture these cells at least 4-5 days prior to initiating the NK Cytotoxicity Assay. Follow the manufacturer's protocols for culturing K-562 cells.

- **K-562 Cell Preparation**

1. Warm working solution of CellTracker dye to 37ºC.
2. While dye is warming, remove K562 cells and media from culture.
3. Centrifuge cells at 300xg for 10 minutes .
4. Resuspend cells in 1 ml X-VIVO® 15 Serum-free Hematopoietic Cell Medium and count cells using Trypan Blue and a hemocytometer.
5. After counting, remove ~1 x 10^6 cells and add to a separate conical.
6. Fill cell suspension to 1 mL with X-VIVO® 15 Serum-free Hematopoietic Cell Medium for a cell concentration of ~1 x 10^6 cells/mL.
7. Add 1 µL working solution of CellTracker Green Dye to the cell conical.
8. Incubate at 37ºC, 5% CO_2 for 1 hour.
9. After incubation with CellTracker Green Dye (from step 7-8), add 2 mL of PBS to the conical and centrifuge at 300xg for 5 minutes at room temperature.
10. After centrifugation, aspirate or decant tubes to remove wash solution.
11. Resuspend cells in 1 mL pre-warmed X-VIVO® 15 Serum-free Hematopoietic Cell Medium.

- **NK Cell Preparation**

12. To prep NK cells for the assay, remove NK cells and media from culture.
13. Centrifuge cells at 300xg for 10 minutes at room temperature.
14. Resuspend cells in 1 mL pre-warmed X-VIVO® 15 Serum-free Hematopoietic Cell Medium and count using Trypan Blue and a hemocytometer.
15. After counting, remove ~1 x 10^6 cells and add to a separate conical.
16. Fill cell suspension to 1 mL with X-VIVO® 15 Serum-free Hematopoietic Cell Medium for a cell concentration of ~1 x 10^6 cells/mL.

- **Cell Staining**

17. After the appropriate preparation of both K-562 and NK cells, make 2 FACS tube for each condition of K562 cells and NK cells at the following ratio:
    a. 1:1 (i.e. 100,000 K-562 cells : 100,000 NK cells).
    b. 1:0 (i.e. 100,000 K-562 cells only. This will be used to account for spontaneous K-562 cell death).

    NOTE: A recommended minimum of 100,000 K-562 cells for FACS analysis should be taken into consideration.
18. Incubate cells for 3 hours at 37ºC, 5% CO_2.
19. After incubation, add 1 µL SYTOX dead cell stain to each tube.
20. Incubate tubes for 15 minutes at 4ºC.
21. After incubation, add 1 mL of PBS to each tube and centrifuge at 300xg for 5 minutes at 4ºC
22. After centrifugation, decant tubes to remove wash solution.
23. Resuspend cells in 1% PFA and analyze via FACS.
24. Assess cells via the % Cell Death of K-562 cells to determine cytotoxic effect of NK cells (increased K-562 % cell death, as compared to the K-562-only control tubes, indicates high NK cell cytotoxic effect).

Ordering information

Catalog no.	Description	Size
2W-501	NK - Human Natural Killer Cells, Cryopreserved, Negative Selection	≥5 million cells
04-418Q	X-VIVO® 15 Serum -free Hematopoietic Cell Medium	1L, complete with L-Glutamine, gentamicin, and phenol red, xenofree
12-702F	RPMI 1640 Medium	500 mL, with L-Glutamine, phenol red

THESE PRODUCTS ARE FOR RESEARCH USE ONLY.

Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

IL-15 (PeproTech P/N 200-15) mentioned is a product from PeproTech.

IL-21 (PeproTech P/N 200-21) mentioned is a product from PeproTech.

CellTracker™ Green CMFDA Dye (Thermo P/N C2925) mentioned is a product from Thermo Fisher Scientific.

SYTOX™ Blue Nucleic Acid Stain (Thermo P/N S11348) mentioned is a product from Thermo Fisher Scientific.

K-562 Cells (ATCC P/N CCL-243) mentioned is a product from ATCC.