

# ImmunoCult™ NK Cell Expansion Kit

## Culture medium kit for expansion of human NK cells

Catalog #100-0711

1 Kit



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## Product Description

ImmunoCult™ NK Cell Expansion Kit has been developed for the in vitro culture and expansion of human natural killer (NK) cells. This kit enables the user to expand NK cells over 14 days of culture, using ImmunoCult™ NK Cell Base Medium, ImmunoCult™ NK Cell Expansion Supplement, and ImmunoCult™ NK Cell Expansion Coating Material.

ImmunoCult™ NK Cell Base Medium is a serum-free and xeno-free medium optimized for the in vitro culture and expansion of human NK cells. This medium contains recombinant and plasma-derived proteins and synthetic components. Growth factors required for the optimal expansion of NK cells have not been added to ImmunoCult™ NK Cell Base Medium; this allows users the flexibility to prepare a medium that meets their requirements.

ImmunoCult™ NK Cell Expansion Supplement is animal component-free (ACF) and contains a combination of ACF recombinant human cytokines formulated to support the culture and expansion of NK cells. It is supplied as a 100X concentrate. ImmunoCult™ NK Cell Expansion Coating Material is ACF and formulated to promote the expansion of NK cells when applied to non-tissue culture-treated cultureware. It is supplied as a 100X concentrate.

## Product Information

The following components are sold as a complete kit (Catalog #100-0711) and are also available for individual sale.

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
ImmunoCult™ NK Cell Base Medium	100-0712	500 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
ImmunoCult™ NK Cell Expansion Supplement	100-0715	5 mL	Store at -20°C.	Stable for 18 months from date of manufacture (MFG) on label.
ImmunoCult™ NK Cell Expansion Coating Material	100-0714	1.5 mL	Store at 2 - 8°C.	Stable for 2 years from date of manufacture (MFG) on label.

## Materials Required but Not Included

PRODUCT NAME	CATALOG #
D-PBS (Without Ca++ and Mg++)	37350
Hausser Scientific™ Bright-Line Hemocytometer	100-1181
Non-tissue culture-treated cultureware	e.g. 38042 (24 wells)
Trypan Blue	07050

## Preparation of Reagents and Materials

### A. ImmunoCult™ NK Cell Expansion Medium

Use sterile technique to prepare ImmunoCult™ NK Cell Expansion Medium (ImmunoCult™ NK Cell Base Medium + ImmunoCult™ NK Cell Expansion Supplement). The following example is for preparing 10 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw ImmunoCult™ NK Cell Expansion Supplement at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing the aliquots, use immediately. Do not re-freeze.

2. Add 100 µL of ImmunoCult™ NK Cell Expansion Supplement to 9.9 mL of ImmunoCult™ NK Cell Base Medium. Mix thoroughly.

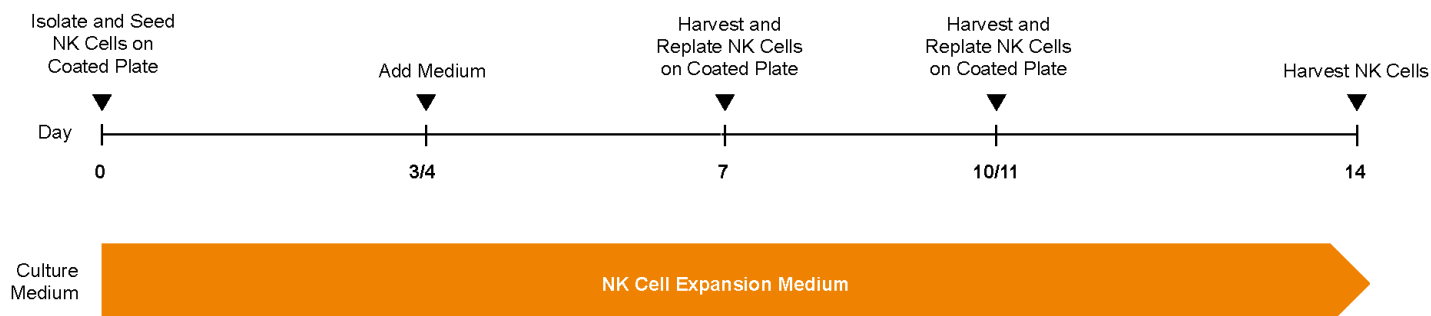
NOTE: If not used immediately, store ImmunoCult™ NK Cell Expansion Medium at 2 - 8°C for up to 4 weeks.

## B. ImmunoCult™ NK Cell Expansion Coating Material (1X)

Use sterile technique to prepare ImmunoCult™ NK Cell Expansion Coating Material (1X) (ImmunoCult™ NK Cell Expansion Coating Material + D-PBS [Without Ca++ and Mg++]). The following example is for preparing 10 mL of coating material. If preparing other volumes, adjust accordingly.

Add 100 µL of ImmunoCult™ NK Cell Expansion Coating Material to 9.9 mL of D-PBS (Without Ca++ and Mg++). Mix thoroughly. Use immediately.

## Protocol Diagram



## Directions for Use

Please read the entire protocol before proceeding. For optimal performance, follow the recommended schedule of feeding and passaging; however, the schedule may be adjusted at each step by +/- one day, as long as the cell density does not become overconfluent and the medium does not change color.

The following protocol is for expansion of NK cells from peripheral blood mononuclear cells (PBMCs), or NK cells isolated from either fresh whole blood or leukapheresis samples. Frozen NK cells may also be used, although expansion will be lower and more variable. The following instructions are for a 24-well plate; if using alternative cultureware, refer to Table 1 for recommended volumes and cell numbers.

**Table 1. Recommended Volumes of Coating Material, Medium, and Seeding Densities for Various Cultureware (Initial Culture)**

NON-TISSUE CULTURE-TREATED CULTUREWARE	VOLUME OF COATING MATERIAL (1X)	VOLUME OF IMMUNOCULT™ NK CELL EXPANSION MEDIUM	NUMBER OF CELLS/WELL (PBMCs or isolated NK cells)	CONCENTRATION OF CELLS (PBMCs or isolated NK cells) (cells/mL)
96-well plate (e.g. Catalog #38044)	100 µL/well	100 µL/well	1 x 10 <sup>5</sup>	1 x 10 <sup>6</sup>
24-well plate (e.g. Catalog #38042)	500 µL/well	500 µL/well	5 x 10 <sup>5</sup>	1 x 10 <sup>6</sup>
12-well plate (e.g. Catalog #38041)	1 mL/well	1 mL/well	1 x 10 <sup>6</sup>	1 x 10 <sup>6</sup>
6-well plate (e.g. Catalog #38040)	2.5 mL/well	2.5 mL/well	2.5 x 10 <sup>6</sup>	1 x 10 <sup>6</sup>

### Day 0

- Add 500 µL of ImmunoCult™ NK Cell Expansion Coating Material (1X) per well of a non-tissue culture-treated 24-well plate.
- Incubate at room temperature (15 - 25°C) for 2 hours.  
NOTE: See Notes and Tips for an overnight coating method.
- Aspirate coating material from the 24-well plate. Rinse the well with D-PBS. Aspirate D-PBS just prior to use.
- Prepare ImmunoCult™ NK Cell Expansion Medium (Preparation section A).
- If using PBMCs, proceed to step 6. If using NK cells, prepare purified NK cells as follows:
  - If using fresh cells, isolate NK cells from fresh (< 24 hours old) human whole blood or from leukapheresis samples using an EasySep™ negative selection kit (e.g. EasySep™ Human NK Cell Isolation Kit [Catalog #17955]). For other EasySep™ kits that may be used, see Notes and Tips. Proceed to step 6.
  - If using frozen NK cells, thaw cells then proceed to step 6.
- Perform a viable cell count using Trypan Blue and a hemocytometer.

7. Add  $5 \times 10^5$  NK cells or PBMCs to 500  $\mu$ L of ImmunoCult™ NK Cell Expansion Medium ( $1 \times 10^6$  cells/mL).
8. Add 500  $\mu$ L of cell suspension (prepared in step 7) to one coated well of the 24-well plate prepared in step 3. Incubate at 37°C and 5% CO<sub>2</sub> for 3 or 4 days.

**Day 3 or 4**

9. Carefully add 500  $\mu$ L of ImmunoCult™ NK Cell Expansion Medium per well of the 24-well plate. Incubate at 37°C and 5% CO<sub>2</sub> for 3 or 4 days.

**Day 7: Harvest and reseed**

10. Prepare a new 24-well plate as described in steps 1 - 3.
11. Gently pipette up and down in the well to ensure all cells are in suspension, then transfer cell suspension to an appropriate tube. Rinse the wells with 1 mL D-PBS and transfer to tube.
12. Centrifuge tube at 300 x g for 10 minutes. Aspirate the supernatant and resuspend the cell pellet in ImmunoCult™ NK Cell Expansion Medium.
13. Perform a viable cell count using Trypan Blue and a hemocytometer.
14. Add  $2 \times 10^5$  cells to 1 mL of ImmunoCult™ NK Cell Expansion Medium ( $2 \times 10^5$  cells/mL). These instructions are for a 24-well plate; if using alternative cultureware, refer to Table 2.

**Table 2. Recommended Volumes of Coating Material, Medium, and Seeding Densities for Various Cultureware (Reseeding)**

NON-TISSUE CULTURE-TREATED CULTUREWARE	VOLUME OF COATING MATERIAL (1X)	VOLUME OF IMMUNOCULT™ NK CELL EXPANSION MEDIUM	NUMBER OF CELLS/WELL (PBMCs or isolated NK cells)	CONCENTRATION OF CELLS (PBMCs or isolated NK cells) (cells/mL)
96-well plate (e.g. Catalog #38044)	100 $\mu$ L/well	200 $\mu$ L/well	$4 \times 10^4$	$2 \times 10^5$
24-well plate (e.g. Catalog #38042)	500 $\mu$ L/well	1 mL/well	$2 \times 10^5$	$2 \times 10^5$
12-well plate (e.g. Catalog #38041)	1 mL/well	2 mL/well	$4 \times 10^5$	$2 \times 10^5$
6-well plate (e.g. Catalog #38040)	2.5 mL/well	5 mL/well	$1 \times 10^6$	$2 \times 10^5$

15. Add 1 mL of cell suspension (prepared in step 14) to one coated well of the 24-well plate prepared in step 10.
16. Incubate at 37°C and 5% CO<sub>2</sub> for 3 or 4 days.

**Day 10 or 11: Harvest and reseed**

17. Prepare a new 24-well plate as described in steps 1 - 3, then harvest and reseed cells as described in steps 11 - 15. Incubate at 37°C and 5% CO<sub>2</sub> for 3 or 4 days.

**Day 14**

18. Gently pipette cells up and down to ensure all cells are in suspension. Transfer cells to an appropriate tube. These expanded cells are ready for assays or analysis as required. For some donors, cell cultures can be extended beyond 14 days for greater expansion of NK cells.

**Optional Extended 21-Day Culture**

19. Prepare a new 24-well plate as described in steps 1 - 3, then reseed cells as described in steps 11 - 15. Incubate at 37°C and 5% CO<sub>2</sub> for 3 or 4 days.

**Day 17 or 18: Harvest and reseed**

20. Prepare a new 24-well plate as described in steps 1 - 3, then harvest and reseed cells as described in steps 11 - 15. Incubate at 37°C and 5% CO<sub>2</sub> for 3 or 4 days.

**Day 21**

21. Gently pipette cells up and down to ensure all cells are in suspension. Transfer cells to an appropriate tube. These expanded cells are ready for assays or analysis as required.

## Notes and Tips

- When coating cultureware with ImmunoCult™ NK Cell Expansion Coating Material (1X), it may be incubated at 2 - 8°C overnight or at room temperature (15 - 25°C) for 2 hours, if desired.
- NK cell expansion varies from donor to donor. The protocol outlined above is optimized for an average NK cell sample. As some donor NK cells may expand faster or slower than expected, careful monitoring of the culture is advisable. Adjusting the harvest and reseeding steps to ensure cells do not overgrow may improve performance.
- Recommended EasySep™ kits for this application use negative selection. These include the following:
  - EasySep™ Human NK Cell Isolation Kit (Catalog #17955)
  - EasySep™ Human NK Cell Enrichment Kit (Catalog #19055)
  - RosetteSep™ Human NK Cell Enrichment Cocktail (Catalog #15025)

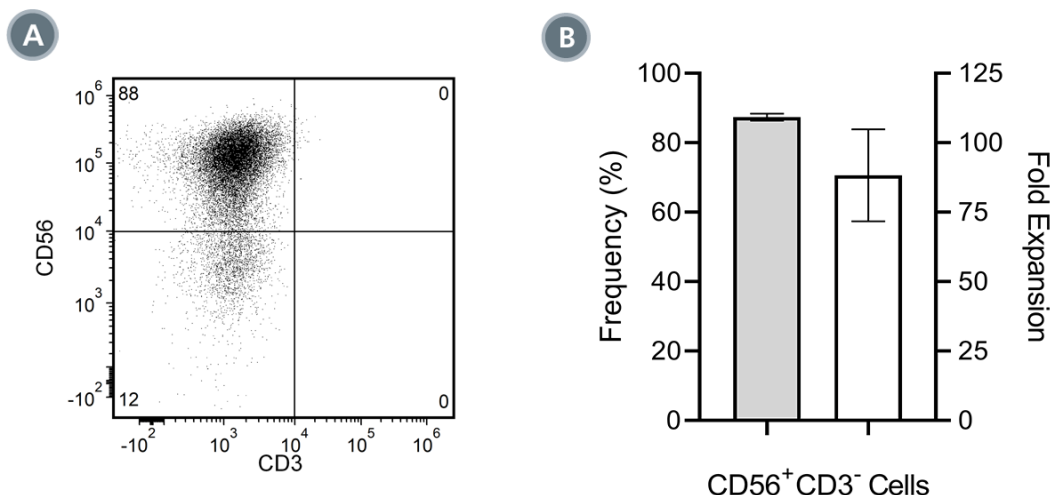
NOTE: EasySep™ Human CD56 Positive Selection Kit II (Catalog #17855) may also be used but may result in additional NK T cell contamination.

- For phenotype assessment of NK cells by flow cytometry, use the following fluorochrome-conjugated antibodies:
  - Anti-Human CD56 Antibody, Clone HCD56 (Catalog #60021)
  - Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011)

Additional fluorochrome-conjugated antibodies for optional analysis:

- Anti-Human CD16 Antibody, Clone 3G8 (Catalog #60041)
- Anti-human CD158 (KIR) antibody, clone 180704 and/or HP-MA4
- Anti-human CD335 (NKp46) antibody, clone 9E2
- Anti-human CD336 (NKp44) antibody, clone P44-8
- Anti-human CD337 (NKp30) antibody, clone P30-15
- Anti-human NKG2D antibody, clone 1D11

## Data



**Figure 1. CD56+ NK Cells Expand in Feeder- and Serum-Free Culture**

CD56+ NK cells, freshly isolated from human PBMCs (leukopak) using EasySep™ Human NK Cell Isolation Kit, were cultured in ImmunoCult™ NK Cell Expansion conditions for 14 days. Cells were harvested and analyzed for expression of CD56 and CD3 by flow cytometry. **(A)** Representative flow cytometry plot. **(B)** The average frequency of viable CD56+CD3- NK cells on day 14 was 87% ± 1%. The average fold expansion of CD56+CD3- cells was 88 ± 17. Results shown represent mean ± SEM (n = 34).

## Related Products

For related products, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit [www.stemcell.com](http://www.stemcell.com), or contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com).

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