PROTOCOL **Aim-NK HMP**
Version 1. 2. 1
STORE IN 2-8°C
Human NK Activation & Expansion Kit
FOR RESEARCH USE ONLY

Other Required Materials

- ○ Cryopreserved or fresh PBMCs, or isolated CD3-CD56+ NK cells
- ○ RPMI 1640 supplemented with 2 mM L-glutamine (or equivalent)
- ○ Heat inactivated Fetal Bovine Serum (FBS)
- ○ HEPES Buffer
- ○ Penicillin/streptomycin (P/S)
- ○ Recombinant human IL-2 (IL-2)
- ○ Recombinant human IL-15 (IL-15)
- ○ Recombinant human IL-18 (IL-18)
- ○ Recombinant human IL-21 (IL-21)
- ○ 2-Mercaptoethanol
- ○ Cell culture vessels
- ○ Humidified CO_2 incubator or bioreactor

Recommended Protocol

1 Medium preparation

Base media

RPMI ● 10% FBS ● 1% P/S ● 50 µM 2-mercaptoethanol ● 25 mM HEPES ● 1X non-essential amino acid

Expansion media

Base media ● 200 U/mL IL-2

Priming media

Base media ● 15 ng/mL IL-21 ● 5ng/mL IL-15 ***or*** 50ng/mL IL-18

Add cytokine cocktail and 2-mercaptoethanol freshly

2 HMP preparation

Resuspend Aim-NK HMP in the vial by vortexing.

3 Priming

We suggest priming **cryopreserved PBMCs** or **NKs** before activation. Prepare **priming media** freshly prior to use. Resuspend the PBMC or NK cells in priming culture media at 2.5×10^5-5×10^5/mL, and prime for **16-24** hours to stimulate NK expressing lost surface ligands on the surface due to cryopreservation. Harvest primed cells and centrifuge at 500g for 5 minutes to discard priming media. Resuspend cell pellet in expansion media, count viable cell number. Dilute cell to 2.5×10^5-5×10^5/mL using expansion media.

Fresh PBMCs or NK can be activated with Aim-NK HMPs directly.

4 HMP and cell seeding

Calculate desired HMP seeding volume per well. Freshly prepare adequate HMP dilutions in culture medium for easy and accurate pipetting. Aliquot diluted HMP into each well. Aliquot resuspended NK cells. Gently mix NK cells and HMP by pipetting up/down 3 to 5 times ensure HMP and cells are evenly distributed under microscope.

Recommendations

- ◇ Cell seeding density : 1×10^5 - 3×10^5 cell per cm²
- ◇ Aim-NK HMP to PBMC/NK ratio : 1:1

Plate	Area	Cell/well	Cell/cm²	1x HMP vol.
96-well	0.32 cm²	8×10^4	2.5×10^5	2 µL
48-well	0.95 cm²	2×10^5	2.1×10^5	5 µL
24-well	1.9 cm²	4×10^5	2.1×10^5	10 µL
6-well	9.5 cm²	2×10^6	2.1×10^5	50 µL
T25	25 cm²	6×10^6	2.4×10^5	150 µL
T75	75 cm²	2×10^7	2.7×10^5	500 µL

Description

Aim-NK hydrogel microparticles (HMP) are designed for robust activation and expansion of human NK cells using a feeder-free culturing approach. For any downstream processing (e.g. counting, flow cytometry) or if earlier removal of Aim-NK HMP is desired, please remove using the digesting buffer provided in the kit.

Components

A suspension of hydrogel microparticles (HMP) made of chemically crosslinked dextran. HMPs sized 8-12 µm were coated with phospholipid bilayer, with membrane docked human NK cell activating signal panels. Each kit contains:

- ◇ One vial of 0.5 mL suspension of AimNK HMP in PBS (4×10^7 beads/mL), with 0.5% P/S, total HMP number = 2×10^7.
- ◇ Two vials of HMP digesting buffer (20X, 1.0mL), containing dextranase to digest HMPs.

Stability and Storage

- ◇ Shipped with ice, keep product refrigerated (2-8°C)
- ◇ Stable at 4°C for 9 months
- ◇ Contents are sterile in unopened tube
- ◇ Do not subject product to freezing, high temperatures (>40°C)

- ◇ When using T-flasks, position them vertically to maximize initial HMP-cell interaction. Flasks can be returned to horizontal position after 2 days once HMP-cell clusters have formed.
- ◇ NK cell growth is donor dependent, especially when starting with PBMC.
- ◇ For experienced users, we recommend optimizing cell seeding density and HMP-to-cell ratio based on your specific requirements. The key principle is to maximize NK cell contact with HMPs during the first 48 hours while avoiding overcrowding.

5 Co-culture

Incubate in a humidified 5% CO_2 incubator at 37°C. Monitor cell morphology and confluency every other day. **DO NOT** disturb HMP-cell interaction in the first 5 days.

Starting from Day 4, perform half-media change every 2 days with **Expansion media**. At Day 6-7, gently pipette cell-AimNK HMP suspensions up and down to break up clumps.

Determine cell density and replate cells to a larger culture vessel if concentration exceeds 2E6 cells/mL or top-up with fresh media to adjust cell concentration to 4×10^5 cells/mL.

6 HMP cleanup

HMP can be rapidly enzymatically degraded by adding the included HMP digesting buffer directly into the culture medium at 0.5-1X final concentration. After incubating at 37°C for 1-2 hours, the HMPs will fully degrade.

7 Restimulation

Aim-NK-stimulated NK cells can maintain proliferation for up to 20 days following a single stimulation, with peak proliferation occurring between Day 5-14, followed by a reduced growth rate thereafter. For enhanced expansion, restimulation may be performed at Day 14. We recommend using a HMP to cell ratio of 1:1 for restimulation protocols.

Things to note

When seeding cells with Aim-NK HMP

Ensure cells and HMP are evenly distributed to maximize interaction.

When you see NK cell cluster

Aim-NK HMP and NK cells tend to aggregate in the well center over time. Gently shake the culture plate to redistribute HMP and NK cells. **Avoid unnecessary pipette mixing in the first 4 days**, disturbing the HMP-cell clustering will cause suboptimal cell growth.

When to add new medium or split?

Monitor cell growth periodically by performing cell sampling and counting. Supplement fresh medium or pass the cells to new culture vessels.

Other compatible media

We have tested Aim-NK compatibility in several commercial NK specific media. Adding Aim-NK in general boosted NK cell activation and growth by 5-10 times.

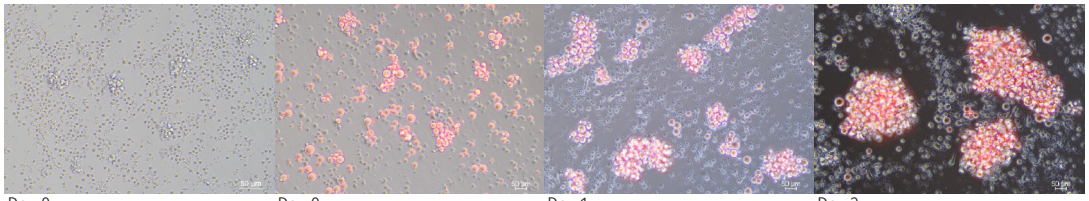
Website

LinkedIn

Email

Representative data

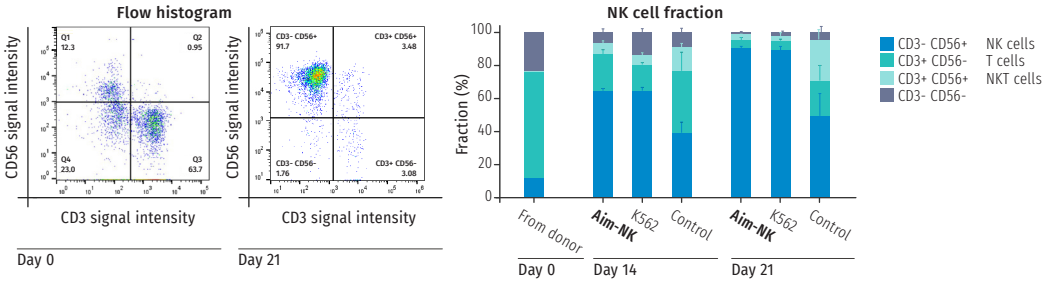
Priming and initial expansion

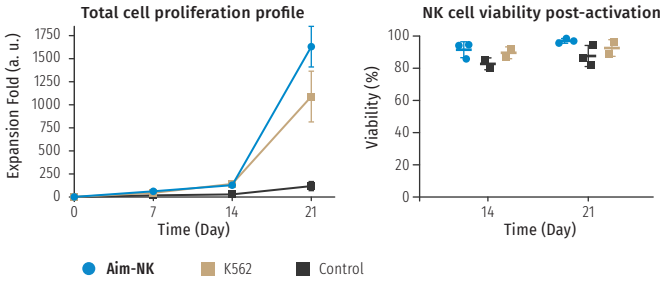


Day 0	Day 0	Day 1	Day 3
PBMC after priming	2 hours post Aim-NK co-culture	24 hours post Aim-NK co-culture	70 hours post Aim-NK co-culture

Representative micrographs showing the activation and expansion of NK cells directly from PBMC at various time points. Scale bar = 50 µm. PBMC were firstly primed with **Priming media** (complete RPMI/10% FBS and IL-18 at 50ng/mL and IL-21 at 15 ng/mL). NK cells were activated and expanded using Aim-NK HMP at 1:1 ratio in **Expansion media** (complete RPMI/10% FBS with 200U/mL IL-2). Red signals indicate the HMPs.

Expansion & activation profile





AimNK AimGel Allegrow

www.allegrowbiotech.com