PROTOCOL

Aim-NK

Human NK Cell Activator

Version 1.0.4
FOR RESEARCH USE ONLY
STORE IN 2-8°C

CATALOG ITEMS: ANK01-0005H; ANK01-0010H; ANK01-0050H

Description

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

Components

A suspension of hydrogel microparticles (HMP) made of chemically crosslinked dextran. HMPs sized 15 µm were coated with phospholipid bilayer, with membrane docked human NK cell activating signal panels. Each kit contains:

- 1 vial of Aim-NK in PBS at 4 x 10^7 beads / mL, with 0.5% P/S
- 1 vial of HMP digesting buffer (100X, 0.5 mL), contains dextranase in PBS

Item	Aim-NK		HMP digesting buffer
ANK01-0005H	0.05 mL	HMP count: 2×10^6	0.1 mL
ANK01-0010H	0.1 mL	HMP count: 4×10^6	0.2 mL
ANK01-0050H	0.5 mL	HMP count: 2×10^7	1.0 mL

Stability and Storage

- Shipped with ice, keep product refrigerated (2-8°C)
- Stable at 4°C for 12 months
- Contents are sterile in unopened tube
- Do not subject product to freezing, high temperatures (>25°C)

Other Required Materials

- ○ Cryopreserved or freshly isolated human PBMC, or isolated $CD3^-CD56^+$ Cells
- ○ RPMI 1640 supplemented with 2 mM L-glutamine (or equivalent)
- ○ Heat inactivated Fetal Bovine Serum (FBS)
- ○ Penicillin/streptomycin
- ○ Recombinant human IL-2 (rIL-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

Activation Protocol

Medium preparation

Base media

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

Priming media

Base media, plus 15 ng/mL IL-21, 5ng/mL IL-15 ***or*** 50ng/mL IL-18. Add cytokine cocktail and 2-mercaptoethanol freshly.

Expansion media

Base media, plus 200-500 U/mL IL-2. IL-2 concentration listed by the culture media is also acceptable.

Priming

Fresh PBMCs or NK can be activated with Aim-NK HMPs directly without priming.

For **cryopreserved PBMCs** or **NKs,** we suggest priming 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 appropriate concentration using expansion media.

HMP preparation

Resuspend HMP in the vial by vortexing for 30 seconds. Open HMP vial and HMP lysis buffer only in sterile environment to avoid contamination. Calculate the desired HMP seeding density per well and aliquot the HMP into each well accordingly. Rather than recommending a fixed bead-to-cell ratio, we provide a validated seeding density for reference. We also encourage experienced users to optimize seeding conditions based on their specific experimental requirements. The HMP seeding principle is to balance the interaction probability between HMP and target cells while leaving sufficient space for cells to grow.

Recommendations

PBMC seeding density : 5×10^5 cell per cm^2
Isolated $CD3^-CD56^+$ NK cell seeding density : 2.5×10^5 cell per cm^2
HMP seeding density : 2.5×10^5 HMP per cm^2

Plate	Area (cm^2)	PBMC/well	NK/well	HMP/well	HMP Vol.
96-well	0.32	1.6×10^5	8×10^4	8×10^4	2.0 µL
48-well	0.95	4.8×10^5	2.4×10^5	2.4×10^5	5.9 µL
24-well	1.9	9.5×10^5	4.8×10^5	4.8×10^5	12 µL
6-well	9.5	4.8×10^6	2.4×10^6	2.4×10^6	60 µL

Plate	Area (cm^2)	PBMC/well	NK/well	HMP/well	HMP Vol.
T25 flask	25	1.3×10^7	6.3×10^6	6.3×10^6	156 µL
T75 flask	75	3.8×10^7	1.9×10^7	1.9×10^7	469 µL

Cell seeding
Aliquot resuspended PBMC or isolated NK cells. Gently mix cells and HMP by pipetting up/down 3 to 5 times ensure HMP and cells are evenly distributed under microscope.

Initial activation
Incubate in a humidified 5% CO_2 incubator at 37°C. Monitor cell morphology and confluency, perform half media replenishment every other day with **Expansion media**. HMP-cell clustering is a typical phenomena you should expect in the first week. **DO NOT disturb** HMP-cell interaction in the first 6 days to allow full activation. At Day 6-7, gently pipette cell & Aim-NK HMP suspensions up and down to break up clumps.

Determine cell density and replate cells to a larger culture vessel if concentration exceeds 2×10^6 cells/mL or top-up with fresh media to adjust cell concentration to 4×10^5 cells/mL.

Restimulation
NK cell growth typically slows after day 16 post-initial activation. Users may consider restimulation to promote continued cell expansion. Restimulating with Aim-NK HMP minimizes activation-induced exhaustion through its mild yet effective stimulation signal. We recommend using **1×10^5 HMP per cm^2** for restimulation.

HMP cleanup
HMP will self-degrade through hydrolysis after approximately 20 days in serum consisting RPMI1640 media. Since the hydrolysis rate is pH-dependent, the actual degradation time may vary depending on the culture medium used.

Alternatively, HMP can be rapidly degraded enzymatically by adding HMP digesting buffer (included in the kit) directly to the culture medium at 1× final concentration. After incubating at 37°C for 30-60 minutes, the HMP will be fully degraded.

Important Note
- HMP digesting buffer contains dextranase, which degrades the HMP core. Do not use this buffer if you plan to restimulate cells, as residual enzyme will degrade newly added HMP unless thoroughly washed out.
- It is recommended to degrade HMP before cryopreservation. Wash the cell pellet at least twice before cryopreservation to remove residual dextranase.

Things to Note
When seeding cells with HMPs
Ensure cells and HMP are evenly distributed to maximize interaction.

When cell cluster appears
Aim-NK HMP and cells tend to aggregate in the well centre over time. Gently shake the culture plate to redistribute HMP and cells. **Avoid unnecessary pipette mixing in the first 6 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 periodically. 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**

AimTconv AimGel
Aim-NK is part of our AimGel artificial cell line-up.

Allegrow
www.allegrowbiotech.com

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