

PROTOCOL

AimGel Dog T Cell Activator

CATALOG ITEMS: ATC01-0050D

RESEARCH USE ONLY
STORE IN 2-8°C

Description

AimGel hydrogel microparticles (HMP) are designed for robust activation and expansion of dog T 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 lysis buffer provided in the kit.

Components

A suspension of hydrogel microparticles (HMP) made of chemically crosslinked dextran. HMPs sized 7-12 μm were coated with phospholipid bilayer, with membrane docked dog T cell activating signal panels. Each kit contains:

- ◆ 1 vial of AimGel Dog T Cell Activator in PBS at 4×10^7 microparticles / mL, with 0.5% P/S
 - ATC01-0050D: 0.5mL, total HMP number = 2×10^7
- ◆ 1 vial of HMP lysis buffer (100X, 1.0mL), contains dextranase.

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 (>40°C)

Other Required Materials

- Cryopreserved or freshly isolated dog PBMC, or isolated CD3+ dog T 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)
- 2-Mercaptoethanol
- Cell culture vessels
- Humidified CO₂ incubator or bioreactor

Activation Protocol

1 Medium preparation

RPMI, 10% FBS, 1% Pen/Strep, 25 μM 2-Mercaptoethanol, 200 U/mL rIL-2

Or any other compatible culture media.

2 HMP preparation

Resuspend HMP in the vial by vortexing for 30 seconds. Open HMP vial only in sterile environment to avoid contamination.

3 HMP seeding

Calculate the desired HMP seeding volume per well. Freshly prepare adequate HMP dilutions in culture medium to facilitate accurate pipetting. Aliquot the diluted HMP into each well. We recommend that experienced users optimize the seeding conditions based on their specific requirements.

Recommendations

- ◆ Isolated T cell, or PBMC seeding density : 2×10^5 - 3×10^5 cell per cm^2
- ◆ HMP to cell ratio : **0.5:1**

Plate	Area (cm^2)	Cell/well	Cell/ cm^2	HMP/well	HMP Vol.
96-well	0.32	8×10^4	2.5×10^5	4×10^4	1 μL
48-well	0.95	2×10^5	2.1×10^5	1×10^5	2.5 μL
24-well	1.9	4×10^5	2.1×10^5	2×10^5	5 μL
6-well	9.5	2×10^6	2.1×10^5	1×10^6	25 μL
T25	25	6×10^6	2.4×10^5	3×10^6	75 μL
T75	75	2×10^7	2.7×10^5	1×10^7	250 μL

4 Cell seeding

Aliquot resuspended T cells. Gently mix T cells and HMP by pipetting up/down 3 to 5 times. Ensure HMP and cells are evenly distributed in the well by visual inspection under a microscope.

5 Co-culture

Incubate in a humidified 5% CO₂ incubator at 37°C. Monitor T cell morphology and confluency every other day. **DO NOT disturb** HMP-cell interaction in the first 4 days.

6 Restimulation

T cell growth typically slows after day 10 post-initial activation. Users may consider restimulation to promote continued cell expansion. Restimulating with AimGel HMP minimizes activation-induced exhaustion through its mild yet effective stimulation signal. The recommended HMP-to-cell ratio for restimulation is **0.25:1**. Experienced users are encouraged to optimize restimulation conditions based on their specific requirements.

Plate	Area (cm ²)	Cell/well	Cell/cm ²	HMP/well	HMP Vol.
96-well	0.32	8×10 ⁴	2.5×10 ⁵	2×10 ⁴	0.5 µL
48-well	0.95	2×10 ⁵	2.1×10 ⁵	5×10 ⁴	1.25 µL
24-well	1.9	4×10 ⁵	2.1×10 ⁵	1×10 ⁵	2.5 µL
6-well	9.5	2×10 ⁶	2.1×10 ⁵	5×10 ⁵	12.5 µL
T25	25	6×10 ⁶	2.4×10 ⁵	1.5×10 ⁶	37.5 µL
T75	75	2×10 ⁷	2.7×10 ⁵	5×10 ⁶	125 µL

7 HMP cleanup

Remove HMP by adding HMP lysis buffer (included in the kit) directly to the culture medium at 1× final concentration. After incubating at 37°C for 30 minutes, the HMP will be fully degraded.

AimGel Dog T Cell Activator ships in a non-hydrolysable formulation. If you need AimGel that clears on its own at a set point, we can formulate it to hydrolyse under your culture conditions.

Important Note

- HMP lysis 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.

Cell Phenotype Characterization

- Resting T cells: smaller in size, round shaped
- Activated T cells: larger in size, irregular

Things to Note

When seeding cells with AimGel HMP

Ensure cells and HMP are evenly distributed to maximize interaction.

When you see cell cluster

AimGel HMP and T cells tend to aggregate in the well centre over time. Gently shake the culture plate to redistribute HMP and T 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 periodically. Supplement fresh medium or pass the cells to new culture vessels when:

- ◊ Colour turns orange yellow (acidic, ~ pH 6.5)
- ◊ T Cell grows to over 3 x 10⁶ cells /mL

When to restimulate

Day 8-10 after previous stimulation.



Part of our AimGel artificial cell line-up.



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Disclaimer

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