

PROTOCOL	AimGel Mouse T cell Activator
RESEARCH USE ONLY STORE IN 2-8°C	CATALOG ITEMS: ATC01-0005M, ATC01-0050M

Description

AimGel hydrogel microparticles (HMP) are designed for robust activation of mouse 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 HMP lysis buffer provided in the kit.

Components

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

- 1 vial of AimGel Mouse T Cell Activator in PBS at 4×10^7 microparticles / mL, with 0.5% P/S
- 1 vial of HMP lysis buffer (100X), contains dextranase in PBS.

Catalogue code	AimGel size		HMP lysis buffer size
ATC01-0005M	0.05 mL	Total HMP count: 2×10^6	0.1 mL
ATC01-0050M	0.5 mL	Total 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
- Do not subject product to freezing, high temperatures (>40°C)

Other Required Materials

- Cryopreserved or freshly isolated mouse PBMC, or isolated CD3+ mouse T Cells
- Medium: RPMI 1640 supplemented with 2 mM L-glutamine, 10% FBS, 25 mM HEPES, 1% Pen/Strep (or preferred antibiotic), 25 μM 2-Mercaptoethanol
- Recombinant mouse or human IL-2 (rIL-2)
- Cell culture vessels
- Humidified CO₂ incubator or bioreactor

Part A) Activation Protocol

1. AimGel preparation

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

2. AimGel seeding

Calculate the desired AimGel seeding volume per well. Freshly prepare adequate AimGel dilutions in culture medium to facilitate accurate pipetting. Aliquot the diluted AimGel 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
- AimGel to cell ratio : **0.5:1**

Plate	Area (cm^2)	Cell/well	Cell/ cm^2	AimGel/well	AimGel 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

3. Cell seeding

Resuspend mouse T cells in complete culture medium with 30 U/mL mouse rIL-2 (or equivalent human rIL-2 concentration)

Seed the cells and add the appropriate volume of **AimGel Mouse T Cell Activator** suspension into your chosen culture vessel according to the guidelines in the above **table**.

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

4. Co-culture

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

5. Restimulation

T cell growth typically slows after day 10 post-initial activation. Users may consider restimulation to promote continued cell expansion. Restimulating with AimGel HMPs minimizes activation-induced exhaustion through its mild yet effective stimulation signal. We recommend restimulating 8-10 days after previous stimulation, at an AimGel-to-cell ratio of **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	AimGel/well	AimGel 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

6. AimGel cleanup

Remove HMP by adding HMP lysis buffer (included in the kit) directly to the culture medium at 1× final concentration (default HMP lysis buffer concentration is 100×). After incubating at 37°C for 30 minutes, the HMP will be fully degraded.

For example, if your culture medium is 1 mL, you will add:

$$\frac{1 \text{ mL}}{100} = 10 \text{ µL of HMP lysis buffer}$$

AimGel Mouse 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 HMPs

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 6.6)
- Cell grows to over 3 x 10⁶ cells /mL

Website



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Email



Part of our AimGel artificial cell line-up.



www.allegrowbiotech.com

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