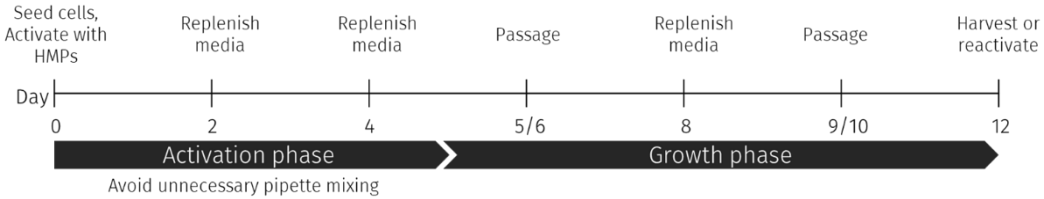
PROTOCOL | **Aim-Tconv Human HMP**

Version 1.1.9
FOR RESEARCH USE ONLY
STORE IN 2-8°C

Human T Cell Activator
CATALOG ITEMS: ATC01-0005H; ATC01-0010H; ATC01-0050H

Description

Aim-Tconv hydrogel microparticles (HMP) are designed for robust activation of human 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 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 T cell activating signal panels. Each kit contains:

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

Item	Aim-Tconv	HMP digesting buffer
ATC01-0005H	0.05 mL HMP count: $2×10^6$	0.1 mL
ATC01-0010H	0.1 mL HMP count: $4×10^6$	0.2 mL
ATC01-0050H	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 (>40°C)

Other Required Materials

- ○ Cryopreserved or freshly isolated human PBMC, or isolated $CD3^+$ 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 CO_2 incubator or bioreactor

Activation Protocol

Medium preparation

RPMI, 10% FBS, 1% Pen/Strep, 20~50 µM 2-Mercaptoethanol, 30~100 U/mL rIL-2
Or any other compatible culture 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 range 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 (seeding density range validated)
Isolated T cell, or PBMC seeding density : $1.5×10^4$ ~ $2.5×10^5$ cell per cm^2
HMP seeding density : $8×10^4$ ~ $1.6×10^5$ HMP per cm^2

Plate	Area (cm^2)	Cell/well	Cell/cm^2	HMP/well	HMP/cm^2	HMP Vol.
96 well	0.32	500~1000		$2.5×10^4$		0.6 µL
		$1.0×10^4$ ~ $8.0×10^4$		$2.5×10^4$ ~ $5.0×10^4$		0.6~1.3 µL
48 well	0.95	$2.4×10^4$ ~ $2.4×10^5$		$7.6×10^4$ ~ $1.5×10^5$		1.9~3.8 µL
24 well	1.9	$4.8×10^4$ ~ $4.8×10^5$		$1.5×10^5$ ~ $3.0×10^5$		3.8~7.6 µL
6 well	9.5	$2.4×10^5$ ~ $2.4×10^6$	$1.5×10^4$~ $2.5×10^5$	$7.6×10^5$ ~ $1.5×10^6$	$8.0×10^4$ ~ $1.6×10^5$	19~38 µL
T25 flask	25	$6.3×10^5$ ~ $6.3×10^6$		$2.0×10^6$ ~ $4.0×10^6$		50~100 µL
T75 flask	75	$1.9×10^6$ ~ $1.9×10^7$		$6.0×10^6$ ~ $1.2×10^7$		150~300 µL

Minimum validated seeding (96-well plate)
Isolated T cells = 500 cells /well
PBMCs = 1,000 cells /well

Cell seeding

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

T cell activation

Incubate in a humidified 5% CO_2 incubator at 37°C. Monitor T cell morphology and confluency, perform half media replenishment every other day. **DO NOT disturb** HMP-cell interaction in the first 4 days.

Restimulation

T cell growth typically slows after day 10 post-initial activation. Users may consider restimulation to promote continued cell expansion. Restimulating with Aim-Tconv HMP minimizes activation-induced exhaustion through its mild yet effective stimulation signal. We recommend restimulating 8-10 days after previous stimulation, and using **8×10^4 HMP per cm^2** for restimulation.

HMP cleanup

HMP will self-degrade through hydrolysis after approximately 10 days in serum consisting of 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 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.

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

Aim-Tconv 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 10^6 cells /mL

Website **LinkedIn** **Email**

AimTconv AimGel

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

Allegrow

www.allegrowbiotech.com

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