

# AimTconv

Human Pan T cell activation and expansion reagent  
**FOR RESEARCH USE ONLY** **STORE IN 2-8°C**



Version 1.1.1

## Description

AimTconv is a suspension of hydrogel microparticles (HMP) designed for robust activation and expansion of human pan T cells. AimTconv HMP are designed to self-degrade in approximately 14 days under culture environment. For any downstream processing (e.g. counting, flow cytometry) and if earlier removal of AimTconv HMP is desired, please remove using the digesting buffer provided in the kit.

## Product Details

<b>Components</b>	<ul style="list-style-type: none"> <li>◇ 1 mL suspension of AimTconv HMP in phosphate buffered saline (PBS) pH 7.4</li> <li>◇ 1 mL AimTconv HMP-digesting buffer at 10X</li> </ul>
<b>Concentration</b>	AimTconv HMP - $4 \times 10^7$ beads/mL
<b>Storage conditions</b>	<ul style="list-style-type: none"> <li>◇ Keep products at <b>2-8°C</b></li> <li>◇ Stable at 4°C for 6 months, contents are sterile in unopened tube</li> <li>◇ Do not freeze or subject product to temperatures higher than 40°C</li> <li>◇ Do not allow AimTconv HMP to dry during storage and use</li> </ul>

## Other Required Materials

### Cultureware

- Tissue culture plate or flask
- Humidified CO<sub>2</sub> incubator or bioreactor

### Media

- RPMI 1640 supplemented with 2 mM L-glutamine, or equivalent culture medium
- Heat inactivated fetal bovine serum (FBS)
- Penicillin/streptomycin
- Recombinant human IL-2 (rIL-2)

## Recommended Protocol

### 1 Medium preparation

RPMI + 10% FBS + 1% Pen/Strep + 30 to 100 U/mL rIL-2, or other compatible culture media.

### 2 AimTconv HMP preparation

Resuspend AimTconv hydrogel microparticles (HMP) in the vial by vortexing.

### 3 AimTconv HMP seeding

Freshly prepare adequate AimTconv HMP dilutions in culture medium for easy and accurate pipetting. Aliquot diluted AimTconv HMP in to each well.

Recommended parameters:

- ◇ Cell seeding density:  $2 \times 10^5$  -  $3 \times 10^5$  cell per cm<sup>2</sup>
- ◇ AimTconv HMP to PBMC ratio 3:1
- ◇ AimTconv HMP to T cell ratio 2:1

### Sample cell seeding plan

Plate	Area (cm <sup>2</sup> )	Cell/well	Cell/cm <sup>2</sup>	2x HMP ratio	3x HMP ratio
96-well	0.32	$8 \times 10^4$	$2.5 \times 10^5$	4 µL	6 µL
48-well	0.95	$2 \times 10^5$	$2.1 \times 10^5$	10 µL	15 µL
24-well	1.9	$4 \times 10^5$	$2.1 \times 10^5$	20 µL	30 µL
6-well	9.5	$2 \times 10^6$	$2.1 \times 10^5$	100 µL	150 µL
T25	25	$6 \times 10^6$	$2.4 \times 10^5$	300 µL	450 µL
T75	75	$2 \times 10^7$	$2.7 \times 10^5$	1000 µL	1500 µL

### 4 Cell seeding

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

## NOTE

Ensure cells and AimTconv HMP are evenly distributed to maximize interaction.

## 5 Co-culture

Incubate in a humidified 5% CO<sub>2</sub> incubator at 37°C. Monitor cell morphology and confluency every other day. **DO NOT** disturb AimTconv HMP-cell interaction in the first 4 days.

### IF YOU SEE CELL CLUSTERING

Cells and AimTconv HMPs tend to aggregate in the well center over time. Gently shake the culture plate to redistribute AimTconv HMP and cells. **Avoid unnecessary pipette mixing in the first 5 days**, disturbing the AimTconv HMP-cell clustering will cause suboptimal cell growth.

Monitor cell growth periodically by performing cell sampling and counting periodically. Supplement fresh medium or pass the cells when:

- ◇ Color turns orange yellow (acidic, ~ pH 6.5)
- ◇ Cell grows to over 3×10<sup>6</sup> cells/mL. Adjust to 0.5-1×10<sup>6</sup> cells /mL.

## 6 AimTconv HMP cleanup

AimTconv HMP will self-degrade by hydrolysis after around 14 days under culture environment. Alternatively, AimTconv HMP can be enzymatically degraded into non-toxic polymeric components using the AimTconv HMP-digesting buffer provided in the kit:

- ◇ Dilute to 1X by adding into a culture media with cells and AimTconv HMP. Gently pipette up and down to mix.
- ◇ Incubate at 37°C for 1-2 hours - AimTconv HMP will degrade completely.

## 7 Restimulation

Restimulation is required for expansion longer than 10 days. Cell growth usually slows down after day 10 after initial activation. Recommended AimTconv HMP to cell ratio for restimulation is 0.5:1 to 1:1, perform **9-11 days** after previous stimulation.

# Cell Phenotype Characterization

## 1 Typical T cell morphology

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

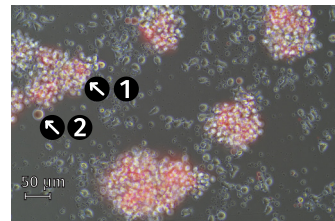
## 2 T cell characterizaion with FACS

	Marker	Type
Lineage	CD3 <sup>+</sup>	Pan T
	CD4 <sup>+</sup>	CD4 T
	CD8 <sup>+</sup>	CD8 T
	CD45RA <sup>+</sup> CD62L <sup>+</sup>	T <sub>naive</sub>
	CD45RA <sup>-</sup> CD62L <sup>+</sup>	T <sub>central memory</sub>
	CD45RA <sup>+</sup> CD62L <sup>-</sup>	T <sub>effector memory</sub>
	CD45RA <sup>-</sup> CD62L <sup>-</sup>	T <sub>effector</sub>
	CD45RO <sup>+</sup>	T <sub>central memory &amp; effector memory</sub>
Activation	CD25 <sup>+</sup>	Activated T
Exhaustion	PD-1 <sup>+</sup>	Exhausted T

## Expansion Profile

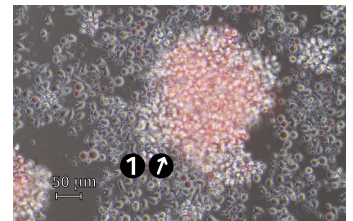
### 1 Human PBMCs co-cultured with AimTconv

Day 2 48WP



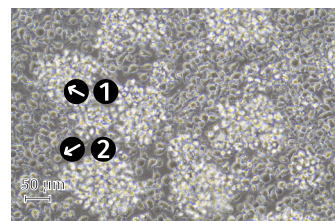
- ① AimTconv HMP-T cell cluster
- ② AimTconv HMP

Day 3 48WP



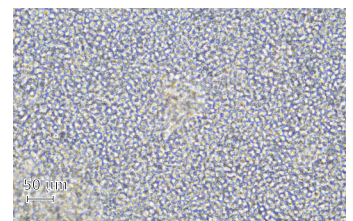
- ① Larger AimTconv HMP-T cell cluster

Day 5 24WP



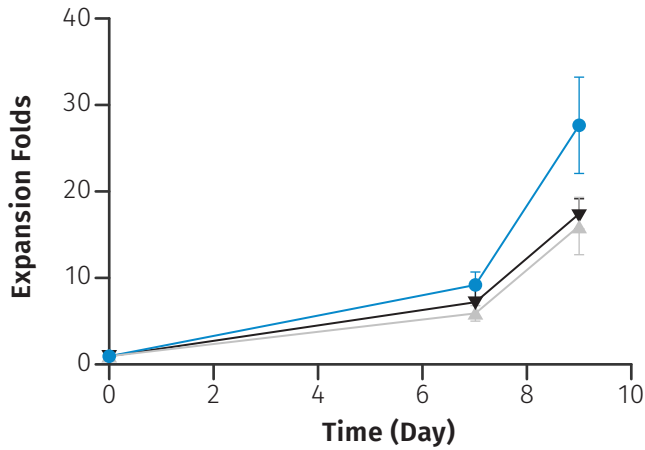
- ① HMPs self-degrade / AimTconv-T cell HMP cluster disassociate
- ② Singular T cell

Day 7 24WP



AimTconv HMPs degrade, release T cells

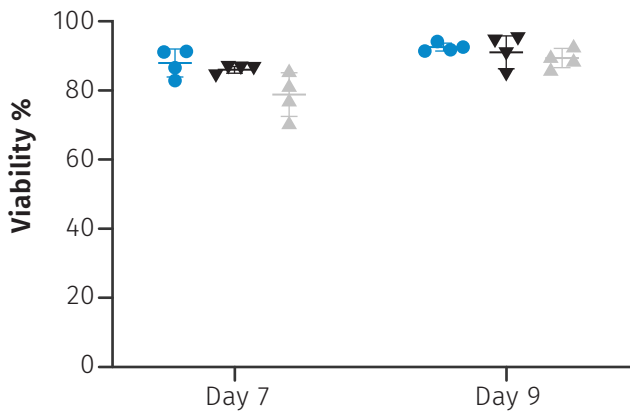
## 2 Cell growth after initial activation



Growth curve of PBMCs after activation by AimTconv HMP at 2:1 HMP:PBMC ratio. Ratios prescribed in the manuals were used for other commercially available reagents.

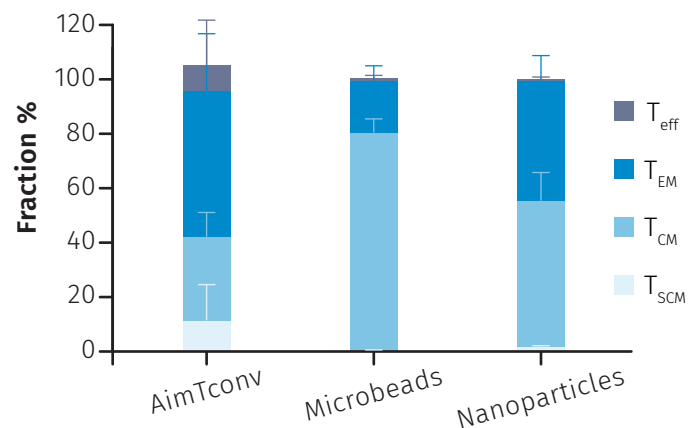
$$\text{Expansion fold} = \frac{\text{viable cells at time } t}{\text{initial number of PBMCs}}$$

## 3 Cell viability after activation



Cell viability of activated PBMC at Day 7 and 9. Cell viability was quantified with trypan blue exclusion assay

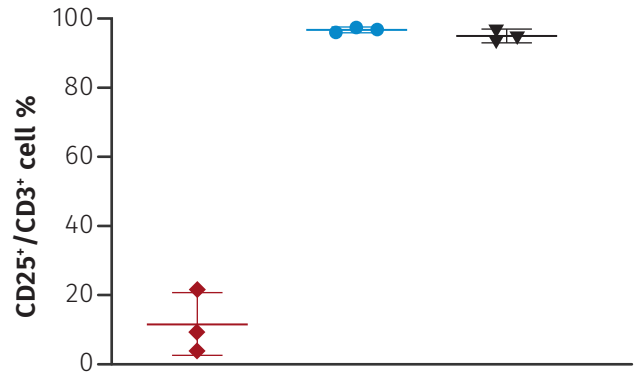
## 4 Memory T cell fraction



AimTconv skews T cells to central and effector memory subtypes

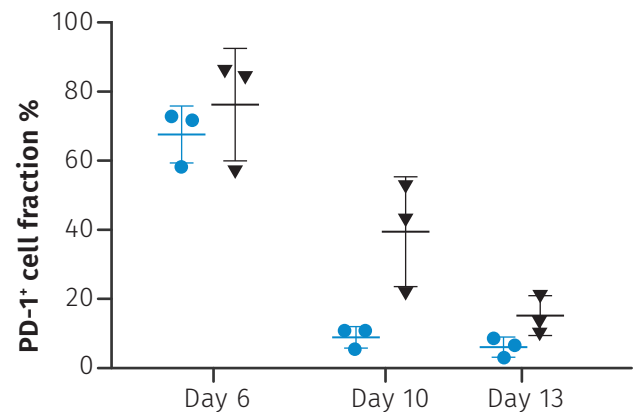
For 2-4, n=4 healthy human donors

## 5 T cell activation on Day 6



Expression of CD25 on CD3<sup>+</sup> cells on Day 6, indicating T cell activation

## 6 Exhausted T cell fraction after activation



Expression of PD-1 on T cells at various time points, indicating T cell exhaustion

- ◆ Untreated
  - AimTconv HMP
  - ▼ Anti-CD3/28 microbeads
  - ▲ Anti-CD3/28 nanoparticles
- For 5-6, n=3 healthy human donors

## Description of Materials

AimTconv is part of our AimGel product line up. The AimGel-platform is composed of self-degrading HMPs made from chemically crosslinked dextran. HMPs sized 5-20µm are coated with lipid membrane that has been functionalized with panel of molecules designed to activate T cells.



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