



Aim-T_{conv} HMP

For human Pan T cell
Activation and Expansion
FOR RESEARCH USE ONLY

Version 1.1.2

Other materials required:

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

Part A) Activation and expansion:

1. **Medium preparation:** RPMI + 10% FBS + 1% Pen/Strep + 20~50 μ M 2-Mercaptoethanol + 30 to 100 U/mL rIL-2, or other compatible culture media.
2. **HMP preparation:** Resuspend Aim-T_{conv} hydrogel microparticles (HMP) in the vial by vortexing.
3. **HMP seeding:** Calculate desired HMP seeding volume per well. Freshly prepare adequate HMP dilutions in culture medium for easy and accurate pipetting. Aliquot diluted HMP in to each well.
 - Recommended T cell seeding density : 2 x 10⁵ to 3 x 10⁵ cell per cm²
 - Recommended HMP to PBMC ratio: 2:1.
 - Recommended HMP to T cell ratio: 1:1.

Example T cell seeding plan				HMP vol. to add	
Plate	Area (cm ²)	T Cell/ well	T Cell/ cm ²	1x HMP ratio	2x HMP ratio
96-well	0.32	8 x 10 ⁴	2.5 x 10 ⁵	2 μ L	4 μ L
48-well	0.95	2 x 10 ⁵	2.1 x 10 ⁵	5 μ L	10 μ L
24-well	1.9	4 x 10 ⁵	2.1 x 10 ⁵	10 μ L	20 μ L
6-well	9.5	2 x 10 ⁶	2.1 x 10 ⁵	50 μ L	100 μ L
T25	25	6 x 10 ⁶	2.4 x 10 ⁵	150 μ L	300 μ L
T75	75	2 x 10 ⁷	2.7 x 10 ⁵	500 μ L	1000 μ L

4. **T 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 under 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. **HMP cleanup:** HMP will self-degrade by hydrolysis after around 14 days under culture environment. Alternatively, HMP can be rapidly enzymatically degraded by adding the included HMP digesting buffer directly into the culture medium at 1X final concentration. After incubating at 37°C for 1-2 hours, the HMPs will be completely degraded
7. **Restimulation:** Restimulation is required for expansion longer than 10 days. T cell growth usually slows down after day 10 after initial activation. Recommended HMP to cell ratio for restimulation is 0.5:1 to 1:1.

Website



allegrowbiotech.com

LinkedIn



allegrow-biotech

Email



welovecells@allegrowbiotech.com

Component Descriptions:

- A suspension of hydrogel microparticles (HMP) made of chemically crosslinked dextran.
- A vial of HMP digesting buffer (10X, 1.0mL), containing enzymes to digest HMP.
- HMPs sized 6-15 μ m were coated with phospholipid bilayer, with membrane docked T cell activating signal panels.
- Supplied at 4 x 10⁷ / mL, in 0.5 mL PBS per vial.
- **Stability and Storage:**
- Shipped with ice, keep product refrigerated (2 to 8°C).
- Stable at 4°C for 6 months. Contents are sterile in unopened tube.
- Do not subject product to freezing, high temperature (greater than 40°C).

Things to note:

When seeding T cells with HMP

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

When you see T cell cluster.

HMP and T cells tend to aggregate in the well center 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 T cell growth periodically by performing T cell sampling and counting periodically. Supplement fresh medium or pass the cells to new culture vessels when

- Color 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.

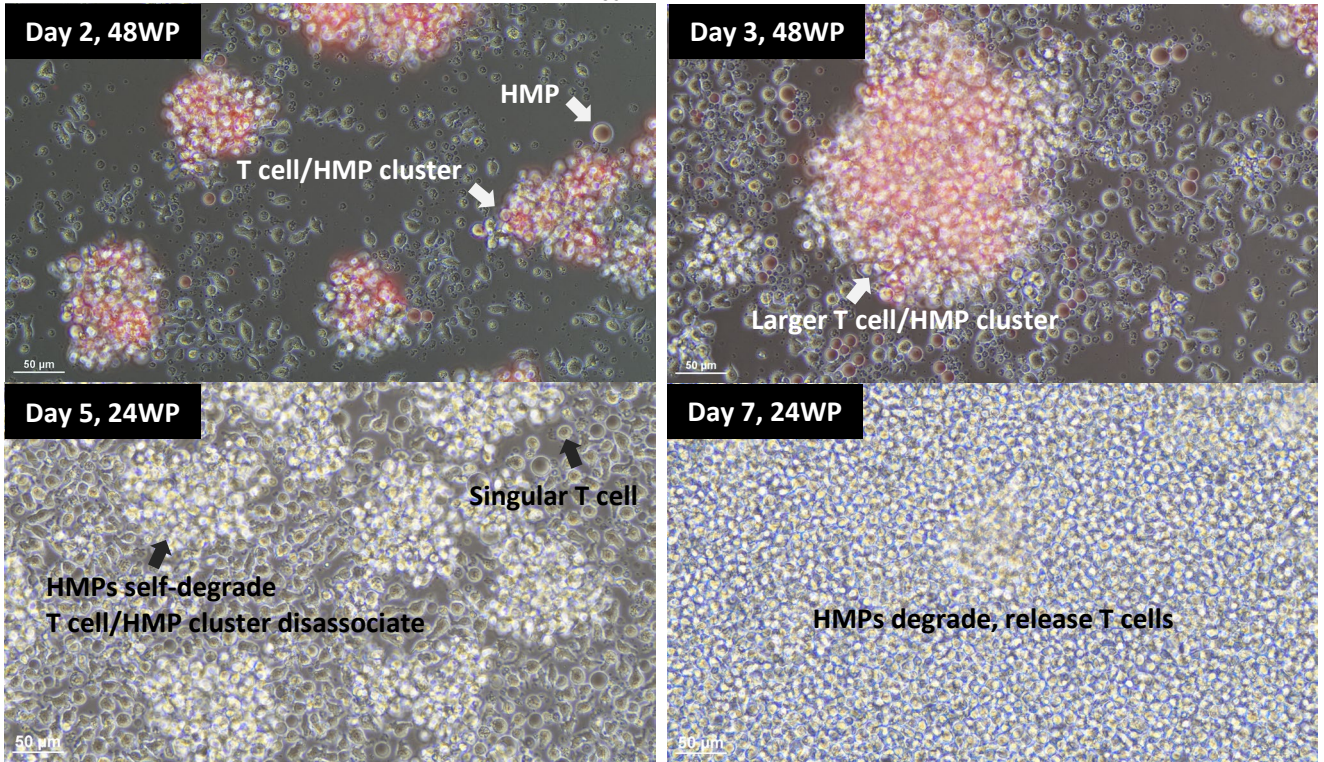
Part B) T Cell phenotype characterization

1. Typical T cell morphology:
 - Resting T cells: smaller in size, round shaped.
 - Activated T cells: are larger in size, irregular.
2. Characterize T cells with FACS accordingly:

	Marker	Type
Lineage	CD3+	Pan T
	CD4+	CD4 T
	CD8+	CD8 T
	CD45RA+ CD62L+	T _{naive}
	CD45RA- CD62L+	T _{central memory}
	CD45RA+ CD62L-	T _{effector memory}
	CD45RA- CD62L-	T _{effector}
	CD45RO+	T _{cm} & T _{em}
Activation	CD25	Activated T
Exhaustion	PD-1	Exhausted T

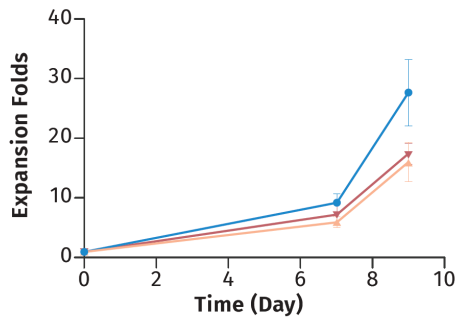
REPRESENTATIVE DATA

Human Pan T cells co-cultured with Aim-T_{conv} at different time points

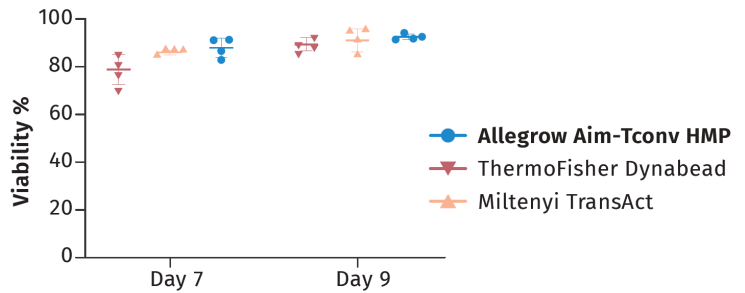


Representative bright-field micrographs showing the activation and expansion of Pan T cells at various time points. Scale bar = 50 μm. Pan T cells were activated and expanded using Aim-Tconv HMP at 1:1 HMP: T cell ratio. Red signals indicate the HMPs.

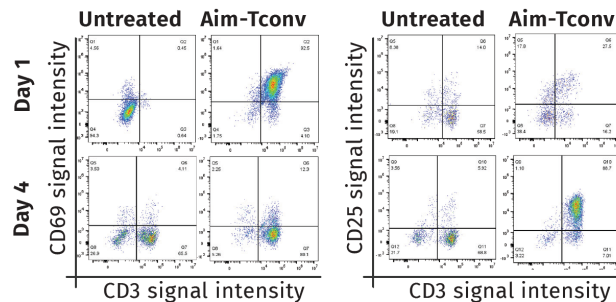
1 T cell proliferation profile



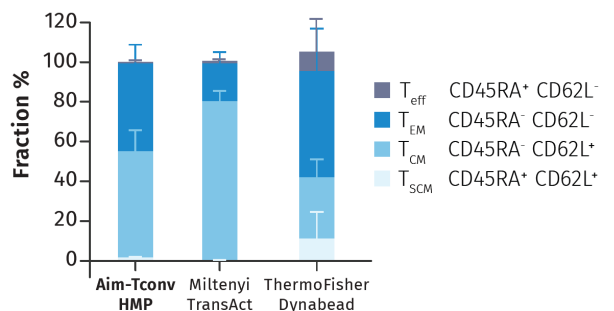
2 T cell viability post-activation



3 T cell activation profiles



4 T cell memory subtypes



1. T Cell growth (n=4 T cells from healthy human donors) after activation by Aim-Tconv HMP and other popular T cell activation reagents. Expansion fold = viable cells at time t / initial number of T cells.
2. T Cell viability (n=4 T cells from healthy human donors) after activation by Aim-Tconv and other popular T cell activation reagents.
3. The expression of early stage marker (CD69) and late stage marker (CD25) indicating T cell activation. Unstimulated T cell was used as gating.
4. T cell memory subtypes after activation by Aim-Tconv and other popular T cell activation reagents.

所需额外试剂：

- ❑ 冷冻保存或新鲜分离的人类泛T细胞（Pan T cell），或已纯化的CD3⁺T细胞。
- ❑ 含2 mM L-谷氨酰胺的RPMI-1640或其他兼容培养基。
- ❑ 热灭活的胎牛血清（FBS）
- ❑ 青霉素/链霉素（P/S）
- ❑ 2-巯基乙醇
- ❑ 重组人 IL-2（rIL-2）
- ❑ 组织培养板或培养瓶
- ❑ 培养箱或生物反应器

A) T细胞的激活与扩增：

- 培养基：** RPMI + 10% FBS + 1% P/S + 20~50 μM 2-巯基乙醇 + 30 to 100 U/mL rIL-2，或其他相容培养基。
- 预备HMP：** 重悬Aim-T_{conv}水凝胶微球(Aim-T_{conv} HMP)。
- 种HMP：** 计算每孔所需的HMP体积，用培养基稀释HMP至适当浓度，分装至培养皿。
 - 建议HMP浓度：每毫升 2 × 10⁵ 至 3 × 10⁵ HMP。
 - 建议HMP与PBMC比例：2:1。
 - 建议HMP与T细胞比例：1:1。
- 种细胞：** 重悬T细胞悬浮液，分装至含有HMP的培养皿中。轻柔地移液混合3至5次。在显微镜下确认HMP与细胞已均匀混合分布。
- 培养扩增：** 将培养皿置于37°C，5% CO₂培养箱孵育。隔天观察细胞形态和覆盖度。加入HMP头四天请勿扰动，避免干扰HMP与T细胞之间的相互作用。
- 清除HMP：**
 - HMP在培养环境可自动水解（约14日）。待HMP完全水解后只需离心清洗。
 - 另外，也可以使用试剂盒中提供的HMP降解剂，将其酶解为无毒的聚合物。需稀释到1X浓度，在37°C条件下孵育1-2小时，HMP将被完全降解。
- 重激活（可选）：** T细胞在扩增10天后生长率会降低。此时可加入新的HMP重新激活。重激活时建议HMP与T细胞比例为0.5:1至1:1。

细胞接种方案				需添加 HMP 体积	
Plate	Area (cm ²)	T Cell/ well	T Cell/ cm ²	1x HMP ratio	HMP ratio
96-well	0.32	8 × 10 ⁴	2.5 × 10 ⁵	2 μL	4 μL
48-well	0.95	2 × 10 ⁵	2.1 × 10 ⁵	5 μL	10 μL
24-well	1.9	4 × 10 ⁵	2.1 × 10 ⁵	10 μL	20 μL
6-well	9.5	2 × 10 ⁶	2.1 × 10 ⁵	50 μL	100 μL
T25	25	6 × 10 ⁶	2.4 × 10 ⁵	150 μL	300 μL
T75	75	2 × 10 ⁷	2.7 × 10 ⁵	500 μL	1000 μL

Website



allegrowbiotech.com

LinkedIn



allegrow-biotech

Email



welovecells@allegrowbiotech.com

本试剂包含以下组分：

- 每管内含 0.5 毫升水凝胶微球(HMP)悬液。
- 每毫升内含 4 × 10⁷ HMP，缓冲液为pH7.4 PBS。
- HMP降解剂(10X), 1.0毫升。
- HMP) 表面覆盖了磷脂膜，膜表面嵌合了多种T细胞激活蛋白信号。

稳定性和存储建议：

- 附冰运输，在 4°C 下可稳定保存 6 个月。
- 未开封的管中内容物经无菌处理。
- 收到产品后，请将其放在冰箱（2 到 8°C）中保存。
- 切勿置于冷冻（低于0°C）或高温（高于 40°C）环境。

注意事项

当开始培养时：

为了使HMP达到最佳激活效率，请在种细胞时充分混合细胞与HMP。

当观察到细胞团簇时：

随着时间的推移，HMP 和细胞倾向于聚集在孔的中心。轻轻摇动培养板以重新分布 HMP 和细胞。

避免在开始后四天内进行移液混合，干扰 HMP-细胞团簇可能会导致细胞死亡。

何时添加新培养基或分盘？

通过定期进行细胞取样和计数来定期监测 T 细胞的生长情况。补充新鲜培养基或转移全部细胞至新培养皿中。

- 当培养基颜色变为橙黄色（酸性条件，约 pH 6.5）。
- 当细胞密度大于每毫升 3 × 10⁶ 细胞，调整至每毫升 0.5-1 × 10⁶ 细胞。

何时需重新激活？

在初期激活后9至11天。

B) T细胞表征：

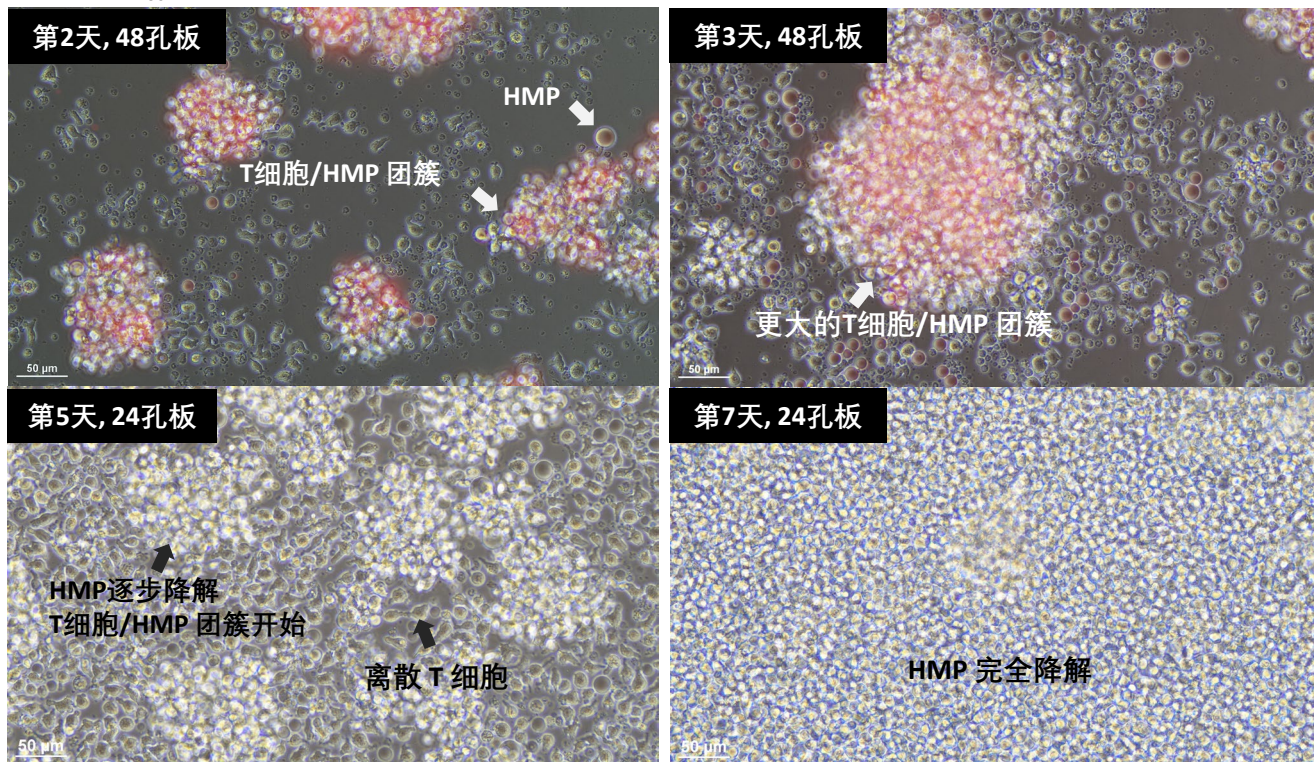
1. 典型T细胞特征：

- 静息T细胞通常体积较小、呈圆形。
- 激活的T细胞体积较大，形状不规则。

2. 用适当的荧光抗体染色细胞，使用流式细胞仪对染色的细胞进行表征：

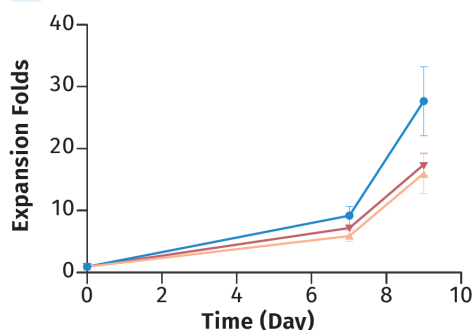
	Marker	Type
Lineage	CD3+	Pan T
	CD4+	CD4 T
	CD8+	CD8 T
	CD45RA+ CD62L+	T _{naive}
	CD45RA- CD62L+	T _{central memory}
	CD45RA+ CD62L-	T _{effector memory}
	CD45RA- CD62L-	T _{effector}
	CD45RO+	T _{cm} & T _{em}
Activation	CD25	Activated T
Exhaustion	PD-1	Exhausted T

使用Aim-T_{conv} 激活人类T细胞后数日内的典型明场显微图

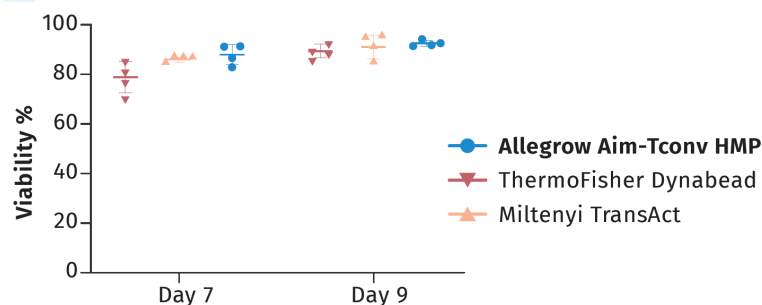


明场显微图，显示了人类广泛T细胞在不同时间点的激活和扩增。刻度 = 50微米。使用 Aim-Tconv HMP 以 1:1 的 HMP：细胞比例激活和扩增T细胞。红色信号代表HMP表面的磷脂膜。

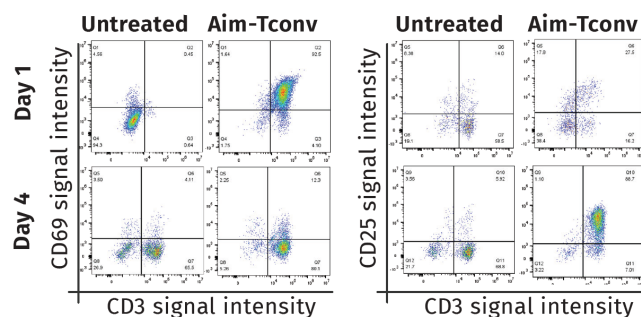
1 细胞生长曲线



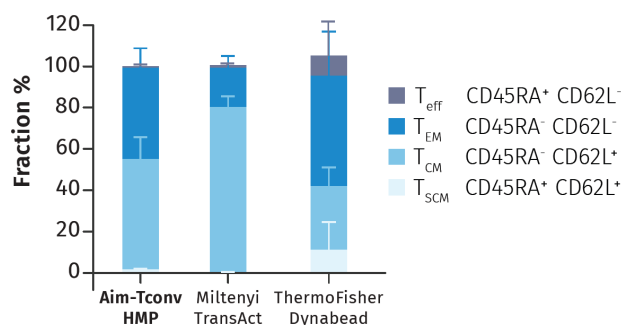
2 激活后不同时间段的细胞活性



3 T细胞激活曲线



4 T细胞记忆亚型



- 使用Aim-Tconv HMP，和其他常用T细胞活化试剂以1:1 HMP:PBMC细胞比例激活后的T细胞增殖曲线对比图。PBMC中只有T细胞能被激活并扩增，扩增倍数= 时间为 t 时，活T细胞数÷初始PBMC细胞总数。
- 使用Aim-Tconv HMP，和其他常用T细胞活化试剂激活细胞后的细胞活性变化对比图。
- 早期标志物（CD69）和晚期标志物（CD25）的表达表明PBMC中的T细胞已激活。
- 使用Aim-Tconv HMP，和其他常用T细胞活化试剂激活T细胞后的T细胞记忆亚型。

* n=4 来自健康人类捐献者，误差条代表均值的1个标准差。