AcnoviaBeads Human T-Activator CD3/CD28

Product Description

AcnoviaBeads Human T-Activator CD3/CD28 (Cat. No. AC68969) are intended for human T cells separation and in vitro expansion.

This can been applied to CAR-T and other T cell culture technologies application.

AcnoviaBeads Human T-Activator CD3/CD28 is composed of 4.5 μm magnetic beads conjugated with anti-human CD3 and anti-human CD28 antibodies，and offers a simple method for isolation and expansion of human T cells. First，AcnoviaBeads Human T-Activator CD3/CD28 enables easy separation and concentration of $CD3^+$ T cells from PBMCs. After isolation, covalent binding of anti-CD3 and anti-CD28 antibodies on magnetic beads provide both the primary and co-stimulatory signals required to regulate T cell activation and expansion. During cell expansion, the cell culture requires additional addition of other cytokines, such as human IL-2, IL-7, and IL-15 for more efficient activation. The activated cell can be expanded 1000-fold over a 10-13 day culture period.

Product Information

Catalog	AC68969
Reactivity	Human
Concentration	2×10^8 beads/mL
Particle size	4.5μm
Endotoxin	<1 EU/mL
Usage	in vitro T cell activation and expansion in enriched T cell or PBMCs
Formulation	phosphate buffered saline (PBS), containing Human Serum Albumin (HSA), pH 7.4.
Stability	24 months
Storage	2 ℃ to 8 ℃, Do not freeze

Product Specifications

Cat. No.	Name	Size	Capacity
AC68969	**AcnoviaBeads Human T-Activator CD3/CD28**	**1mL/10mL**	**For isolation: 6.6×10^7 $CD3^+$ T cells;** **For activation: 2×10^8 enriched $CD3^+$T cells ;**

Materials Required

- Human T cell culture media.
- Human cytokines for optimal expansion, such as, IL-2, IL-7, IL-15.

Protocol

Wash AcnoviaBeads Human T-Activator CD3/CD28

1. Resuspend the AcnoviaBeads Human T-Activator CD3/CD28 (beads) in the tube (vortex for >30 sec, or tilt and rotate for 5min).

2. Transfer the beads with a desired volume into a tub.

3. Add equal volume of PBS (containing 1% HSA). If the beads volume is less than 1mL, add 1mL PBS (containing 1% HSA) for resuspension.

4. Place the tube on the magnet for 1min, and then discard the supernatant.

5.Remove the tube from the magnet, then use the same volume of PBS (containing 1% HSA) to resuspend the beads.

Separate $CD3^+$ T cells

1. For Ficoll isolated PBMCs, gently resuspend the cells in PBS (containing 1% HSA), and adjust the cell density to 2-5×10^7 cells/mL. Note that the total number of cells should not exceed 2×10^8 cells/mL.

Note: Before you begin, determine the percentage of $CD3^+$ T cells in the sample by flow cytometry.

2. Add washed beads into the PBMCs in a ratio of 3 (beads):1 ($CD3^+$ T cell), if the cells are pure isolated T cells, the ratio of beads to cells is adjusted to 1:1.

3. Rotate the samples with a speed of 50~120 rpm/min, and incubate them at room temperature for 30 min.

4. Dilute the mixture of beads and cells with T cell culture media or PBS (containing 1% HSA) to ensure the separation volume for magnetic selection.Following this, place the tube on the magnet for 1~2min.

5. Remove the supernatant, then resuspend the mixture of beads and cells with T cell culture media containing 300 IU/mL IL-2, and adjust the cell density to 0.5×10^6 cells/mL~1×10^6 cells/mL.

6.Put the cell suspension in the incubator with 37 ℃, 5% CO_2.

T cell activation and expansion

1. Count the number of T cells daily, beginning on day 3 of culture.

2. At the later stage of cell expansion, gently blow the cell suspension in the culture regularly to dissociate the beads and cells.

3. When $CD3^+$ T cells >1×10^6 cells/mL, add T cell culture media containing IL-2 to dilute cells, and adjust the cell density to about 0.5× 10^5 cells/mL.

4.At the end of culture (day 9-14), count the cells and remove the beads with magnets.

Performance Data

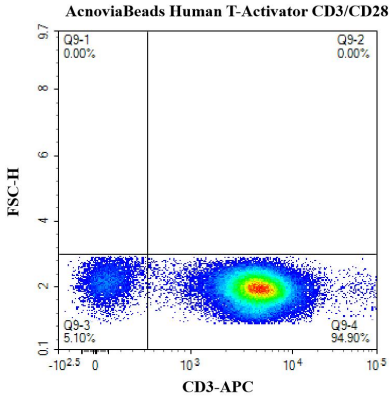

Fig.1 Magnetically separate $CD3^+$ T cells. PMBCs were incubated for 30 min with AcnoviaBeads Human T-Activator CD3/CD28 (Cat. No# AC68969) at a ratio of 3 beads per cell and the positive (isolated) fraction was analyzed isolation efficiency by flow cytometry.

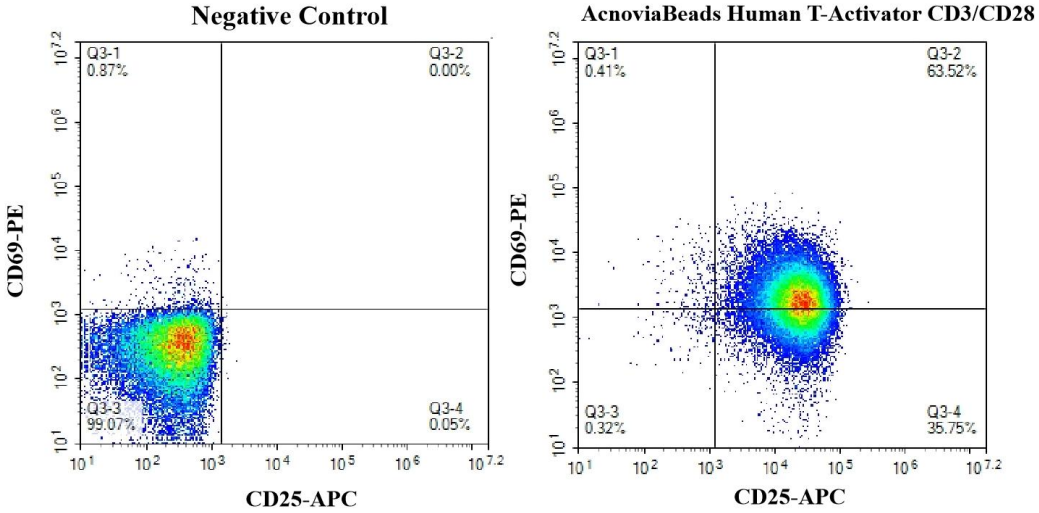

Fig.2 Activation of the purified human $CD3^+$ T Cells. The purified human $CD3^+$ T cells were activated using AcnoviaBeads Human T-Activator CD3/CD28 (Cat. No # AC68969) for 48 hours. Cells were fluorescently stained using APC labeled anti-human CD25 antibody and labeled PE anti-human CD69 antibody, and analyzed by flow cytometry.

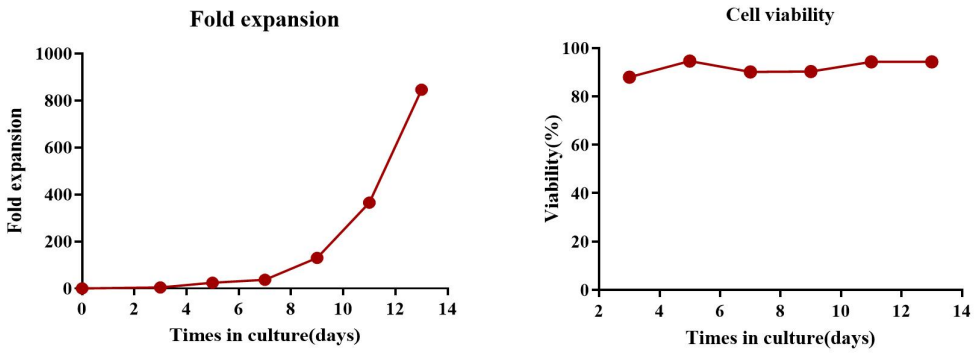

Fig.3 Purified human CD3$^+$T Cells expansion. The purified human CD3$^+$T cells were stimulated using AcnoviaBeads Human T-Activator CD3/CD28 (Cat. No # AC68969). Cells were expanded in T cell culture medium containing 300IU/mL of IL-2. Activated Cells were expanded for up to 13 days (A) with high cell viability (B).