



AcnoviaBeads Human T-Activator CD3/CD28

Product Description

AcnoviaBeads Human T-Activator CD3/CD28 (Cat. No. AC68951) are intended for human T cells separation and in vitro expansion. This can be 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	AC68951
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 °C to 8 °C, Do not freeze

Product Specifications

Cat. No.	Name	Size	Capacity
AC68951-1	AcnoviaBeads Human T-Activator CD3/CD28	1mL	For isolation: 2×10 ⁸ CD3 ⁺ T cells; For activation: 1×10 ⁹ enriched CD3 ⁺ T cells ;
AC68951-1	AcnoviaBeads Human T-Activator CD3/CD28	10mL	For isolation: 2×10 ⁹ CD3 ⁺ T cells; For activation: 1×10 ¹⁰ 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.

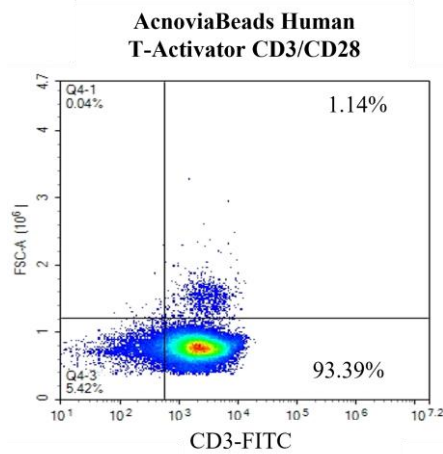
Separate CD3⁺ T cells

1. For Ficoll isolated PBMCs, gently resuspend the cells in PBS (containing 1% HSA), and adjust the cell density to $2-5 \times 10^7$ 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 °C, 5% CO₂.

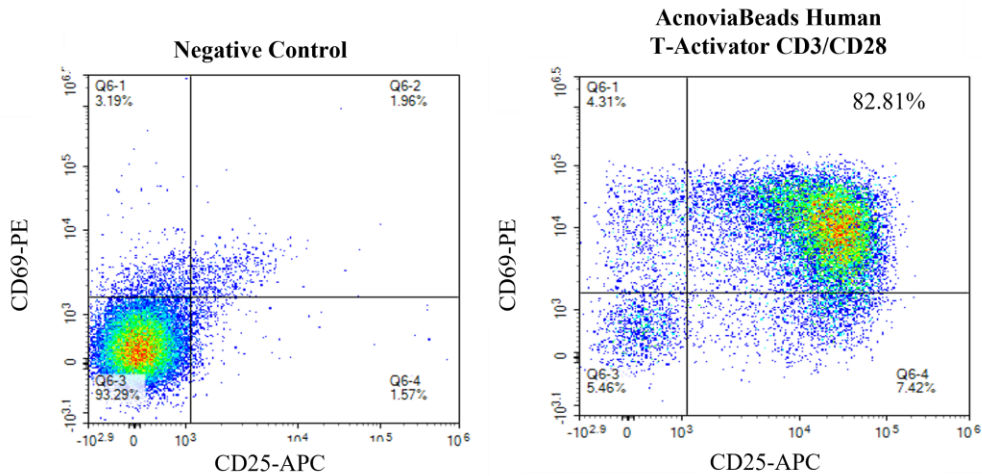
T cell activation and expansion

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 $> 1 \times 10^6$ 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.

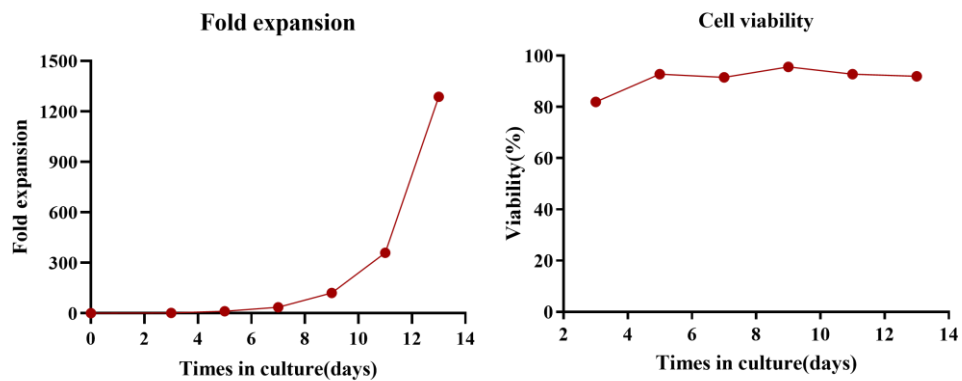
Performance Data



PMBCs were incubated for 30 min with AcnoviaBeads Human T-Activator CD3/CD28 (Cat. No# AC68951) at a ratio of 3 beads per cell and the positive (isolated) fraction was analyzed isolation efficiency.



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 # AC68951) for 48 hours. Cells were fluorescently stained using APC labeled anti-human CD25 antibody and PE labeled anti-human CD69 antibody, and analyzed by flow cytometry.



Purified human CD3⁺ T Cells expansion. The purified human CD3⁺ T cells were stimulated using AcnoviaBeads Human T-Activator CD3/CD28 (Cat. No # AC68951). Cells were expanded in T cell culture medium containing 300 IU/mL of IL-2. Activated Cells were expanded for up to 13 days (A) with high cell viability (B).