# Human T-Activator CD3/CD28, Beads

Cat #: C-BETA2001

Size: 1mL / 10mL

Storage: 2°C to 8°C, Do not freeze

## Product Description

Human T-Activator CD3/CD28, Beads 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.

Human T-Activator CD3/CD28, Beads 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, Human T-Activator CD3/CD28, Beads 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

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| **Catalog** | C-BETA2001 |
| **Reactivity** | Human |
| **Concentration** | $2 \times 10^8$ beads/mL |
| **Particle size** | 4.5 µm |
| **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 |

## Product Specifications

| Cat. No. | Name | Size | Capacity |
|---|---|---|---|
| C-BETA2001 | Human T-Activator CD3/CD28, Beads | 1 mL/10 mL | For isolation: 6.6 × $10^7$ $CD3^+$ T cells; For activation: 2 × $10^8$ enriched $CD3^+$ T cells ; |

## Materials Required

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

## Protocol

### Wash Human T-Activator CD3/CD28, Beads

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

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 1~2 min.

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 × $10^6$ 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.

## Product Data

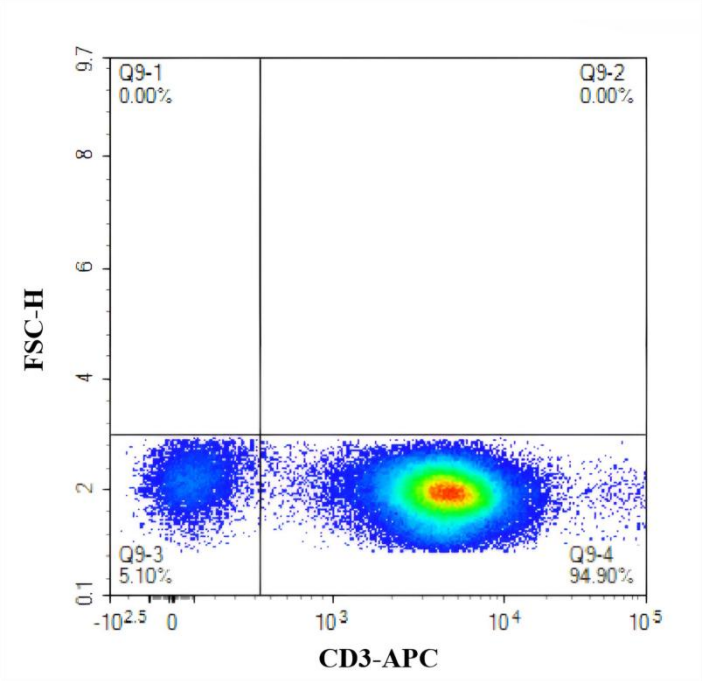


Fig.1 Magnetically separate $CD3^+$ T cells. PMBCs were incubated for 30 min with Human T-Activator CD3/CD28, Beads (Cat. No# C-BETA2001) at a ratio of 3 beads per cell and the positive (isolated) fraction was analyzed isolation efficiency by flow cytometry.

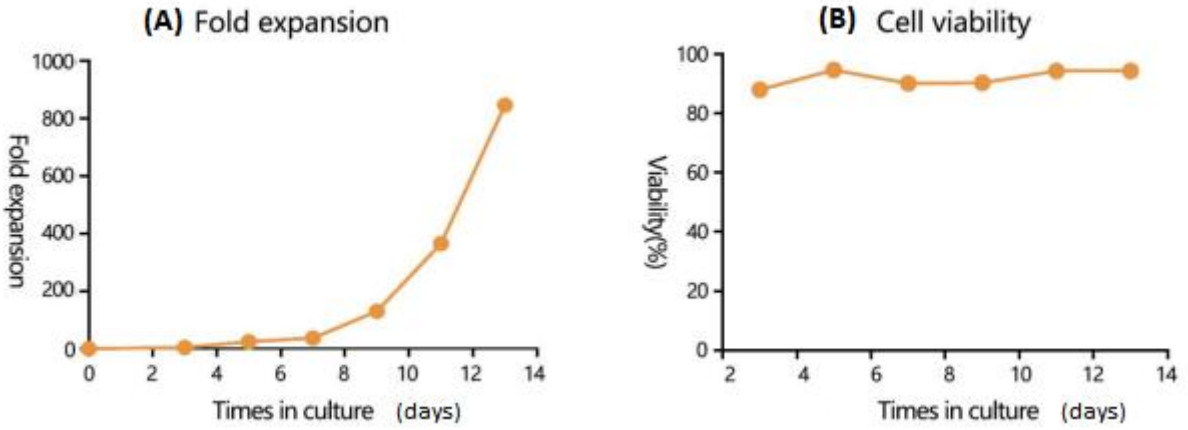


Fig.2 Purified human $CD3^+$T Cells expansion. The purified human $CD3^+$ T cells were stimulated using Human T-Activator CD3/CD28, Beads (Cat. No # C-BETA2001). Cells were expanded in T cell culture medium containing 300 IU/mL of IL-2. Activated Cells were expanded for up to 13 days (A) with high cell viability (B).