

Human T-Activator CD3/CD28, Beads

Cat #: C-BETA2001

Size: 0.5mL / 1mL

Storage: 2°C to 8°C, Do not freeze

Product Description

Human T-Activator CD3/CD28, Beads are intended for human T cell activation and expansion in enriched T cells or PBMCs. Human T-Activator CD3/CD28, Beads is composed of biodegradable matrix-coated nanoparticles conjugated with mouse anti-human CD3 and mouse anti-human CD28 antibodies. The small sized particulate structure of the activation reagent enables its easy removal by media washing or centrifugation.

The recommended titer of 1:200 has been found to achieve efficient stimulation of majority of T cell subsets. For special applications, the optimal stimulation titer should be determined experimentally. Excessive amount of the Human T-Activator CD3/CD28, Beads may result in over-activation of T cells risking activation-induced cell death. Typically, the cells reach the exponential growth phase over 5 days and keep optimal expansion up to 13 days. Restimulation after 13 days can be performed for longer cultivation. Human T-Activator CD3/CD28, Beads can be used together with human IL-2, other cytokines such as human IL-15 and IL-7 can also be added for more efficient activation.

Product Information

Catalog	C-BETA2001
Reactivity	Human
Usage	T cell activation and expansion in enriched T cells or PBMCs
Formulation	Supplied in phosphate-buffered saline (PBS), containing Human Serum Albumin (HSA) and Poloxamer 188.

Product Specifications

Cat. No.	Name	Size	Capacity
C-BETA2001	Human T-Activator CD3/CD28, Beads	0.5 mL/ 1 mL	0.5 mL - Up to 1×10^8 T cells ; 1 mL - Up to 2×10^8 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

Notes:

- All procedures are to be performed under sterile conditions.
- Optimal initial stimulation requires 2 - 3 days of reagent interaction; earlier removal may result in insufficient T-cell proliferation.
- T cells can be transduced 1 - 2 days after activation. The optimal transduction viral titer and timing need to be determined experimentally.
- Residual EDTA can hamper T cell stimulation and needs to be removed before.

1. Sample Preparation

To prepare blood samples for T cell activation, PBMCs should be isolated by density gradient centrifugation.

2. T Cell Activation and Expansion

This protocol has been optimized for gentle and efficient activation and expansion of enriched T cell populations using a titer of 1:200. Purified T cells should be activated at an optimal surface density of 1×10^6 cells per cm^2 (Table 1) PBMCs should be activated at an optimal surface density of 2×10^6 per cm^2 .

Culture plate	Growth area	Culture volume	T cell number (double for PBMC)	Enceed T cell Activation	Culture media
96 well	0.31 cm ²	0.2 mL	0.3×10^6	1 μ L	199 μ L
48 well	1 cm ²	1 mL	1×10^6	5 μ L	995 μ L
24 well	2 cm ²	2 mL	2×10^6	10 μ L	1990 μ L
12 well	4 cm ²	4 mL	4×10^6	20 μ L	3980 μ L
6 well	10 cm ²	5 mL	5×10^6	25 μ L	4975 μ L

Table 1. Optimal surface density

Volumes given below are for stimulation in 48-well plates. 5 μ L of Enceed T cell Activation reagent is effective for up to 1×10^6 purified T cells or up to 2×10^6 PBMCs in a total volume of 995 μ L culture media.

When working with fewer than 10^6 cells, continue using the volumes indicated in Table 1. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly. Irrespective of the culture vessel used, use 5 μ L of Enceed T cell Activation reagent for activation of 1×10^6 purified T cells or 2×10^6 PBMCs.

a. Activation in 48-Well Plates

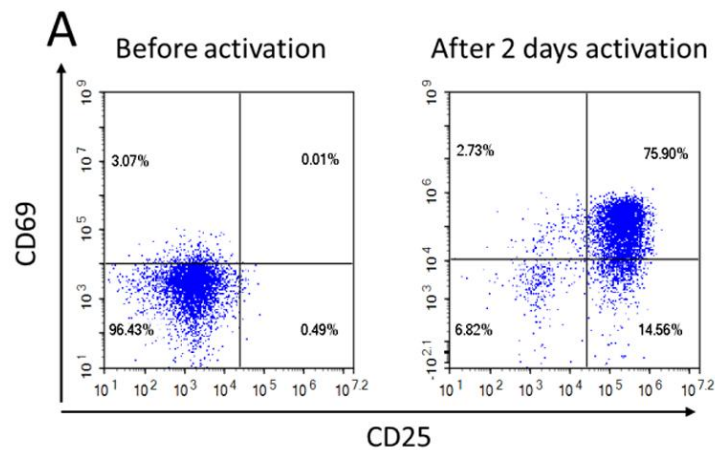
1. Determine the cell number.
2. Resuspend the cells in 995 μ L culture media.
3. Add 5 μ L Enceed T cell Activation reagent.
4. Incubate at 37°C, 5% CO₂ for up to 3 days.
5. Refresh T cell culture media 2 - 3 days after initial activation by adding new media or replacing the media using centrifugation:
 - i. Centrifuge the cell culture at $300 \times g$ for 10 minutes.
 - ii. Aspirate the supernatant completely.
 - iii. Add culture media.
 - iv. Incubate at 37°C, 5% CO₂.

b. Expansion

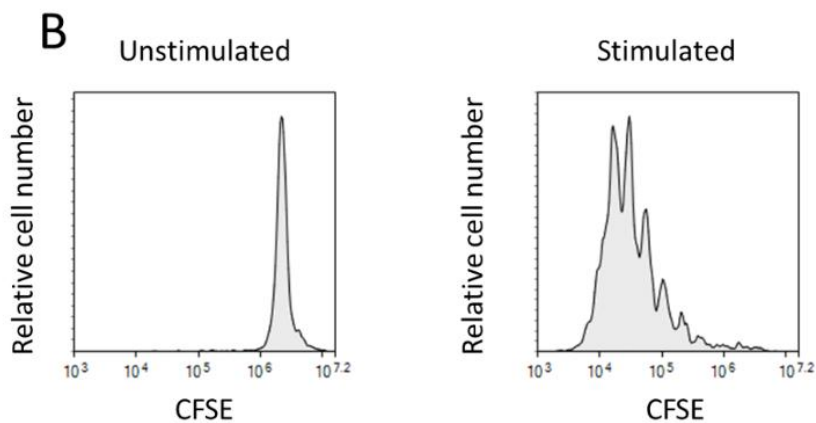
1. Split the cell suspension every 2 days into two equal parts and add culture media.
2. Incubate at 37°C, 5% CO₂.
3. On day 13, the cells are sufficiently expanded for downstream applications.

4. If needed, the time for expansion may be extended to 17 days, depending on the cell state and experimental requirements.
5. If needed, T cells can be restimulated after 13 days by reapplying Enceed T cell Activation reagent.

Product Data



After 2 days, enriched T cells were stained with CD25-PE and CD69-APC antibodies and analyzed by flow cytometry (Figure A).



T cells stained with carboxyfluorescein succinimidyl ester (CFSE) were stimulated by Human T-Activator CD3/CD28, Beads. After 7 days, proliferation was tested by flow cytometry (Figure B).