

Human T Cell Activation Kit (Anti-CD3/CD28)

Catalog Number: RK40100

Size: 1 Box

◆ Description

The Human T Cell Activation Kit (Anti-CD3/CD28) allows for convenient in vitro activation and proliferation of human T cells without the need for antigen-presenting cells. The CD3 molecules non-covalently bind to TCR (T cell Receptor), forming TCR/CD3 complex, which works with CD28 antibodies to mimic the activation effect of APCs (Antigen-Presenting Cells) on T cells. As a result, the CD3/CD28 co-stimulatory signal promotes T cell proliferation and enhances the functional response of T cells.

◆ Applications

FC, in vitro T cell stimulation/activation

◆ Reactivity

Human

◆ Components

Target	Catalog No.	Size	Antibody usage
CD3	A25761	100µg/1mg/5mg/25mg/50mg/100mg	2 µg/mL
CD28	A27700	100µg/1mg/5mg/25mg/50mg/100mg	2 µg/mL

◆ Cell Preparation

1) Collect cells of interest and pellet by centrifugation. PBMCs, enriched T cells, or cell lines may be used. Recommended cell numbers and volumes in every step are identical to pre-isolated T cells.

2) Resuspend cells in cell culture medium at a concentration of 0.2×10^6 - 1×10^6 cells per 1 mL (optimal conditions should be titrated).

◆ T cell activation

1) Coat the activated T cell wells with the anti-CD3 antibody by diluting the anti-CD3 antibody at 2 µg/mL in sterile PBS. Add diluted antibody to the wells at 0.5 mL/well. Incubate plate at 5% CO₂ at 37°C for 2 hours.

2) Prepare complete RPMI 1640 medium by supplementing RPMI 1640 medium with fetal bovine serum to a final concentration of 10% and 2 mM L-glutamine (if using medium not currently supplemented with GlutaMAX). Bring medium to 37 °C. Optional: Supplement media with 1% penicillin-streptomycin (5,000 units/mL).

3) Into the activated tube, add 2 µg/mL of anti-CD28, and set aside in culture hood (room temperature).

4) Retrieve 24-well plate from incubator after 2 hours. Aspirate out the anti-CD3 solution and discard. Do not wash the plate.

5) Divide activated cell solution (from step 3) evenly into the anti-CD3 treated wells (activated T cells) of the 24-well plate (1 mL per well).

6) Incubate culture plate for 1 - 3 days in 5% CO₂ incubator at 37°C. By day 1, cells will

produce transcription factors and cytokines. Most T cells will require up to 3 days to divide. These cells will clump but will not be attached to the plate.

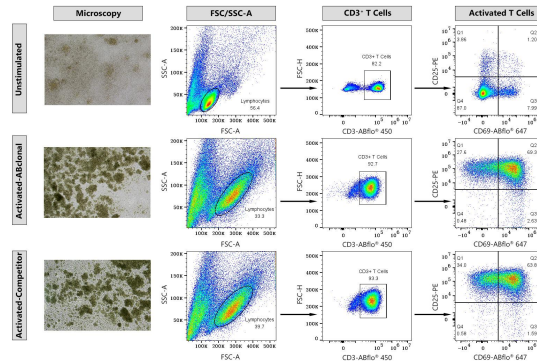
◆ Protocol tips

- 1) IL-2 stimulates the growth and differentiation of cytotoxic T cells. Adding 100 ng IL-2 recombinant protein can help expand this population.
- 2) Optimal concentration range of antibodies anti-CD3 and anti-CD28 should be determined empirically. For anti-CD3 (human), optimal concentration range is 1-3 µg/mL and for anti-CD28 (human), optimal concentration range is 1-3 µg/mL.

◆ Storage

Store at 2-8°C. Avoid freeze. | Buffer: 0.2 µm filtered in phosphate-buffered solution, pH 7.2, containing no preservative. | Endotoxin Level: Less than 0.01 EU/µg of the protein (< 0.001 ng/µg of the protein) as determined by the LAL test.

◆ Validation Data



Human T cells in PBMC were activated using Mouse anti-Human CD3 mAb (A25761,2 $\mu\text{g/mL}$) and Rabbit anti-Human CD28 mAb (A27700,2 $\mu\text{g/mL}$), or competitor's CD3/CD28 antibody for 72 hours. The unstimulated control was performed without treatment. Cells were fluorescently stained with ABflo® 450 Rabbit anti-Human CD3 mAb (A27177,5 $\mu\text{L/Test}$), PE Rabbit anti-Human CD25 mAb (A26773,5 $\mu\text{L/Test}$), ABflo® 647 Rabbit anti-Human CD69 mAb (A25892,5 $\mu\text{L/Test}$). The degree of T cell activation are defined as CD25⁺CD69⁺ cells on CD3⁺ T cells gate.