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

Catalog Number: RK40101

Size: 1 Box

◆ Description

The Mouse T Cell Activation Kit (Anti-CD3/CD28) allows for convenient in vitro activation and proliferation of mouse 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

Mouse

◆ Components

Target	Catalog No.	Size	Antibody usage
CD3	A27913	100μg/1mg/5mg/25mg	2 μg/mL
CD28	A27914	100μg/1mg/5mg/25mg	2 μg/mL

◆ Cell Preparation

1) Collect cells of interest and pellet by centrifugation. Splenocytes, 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 x 10^6 – 1 x 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% CO_2 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 6-well plate (1 mL per well).

6) Incubate culture plate for 1 – 3 days in 5% CO_2 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

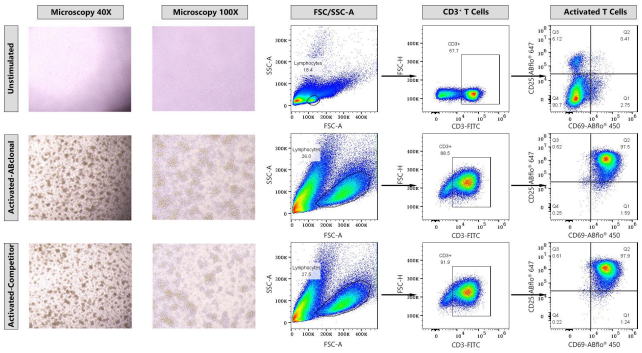
1) IL-2 stimulates the growth and differentiation of cytotoxic T cells. Adding 100 ng IL-2 recombinant protein can help expand this population.

2) Optimal concentration range of antibodies anti-CD3 and anti-CD28 should be determined empirically. For anti-CD3 (mouse), optimal concentration range is 1–3 μg/mL and for anti-CD28 (mouse), optimal concentration range is 1–3 μg/mL.

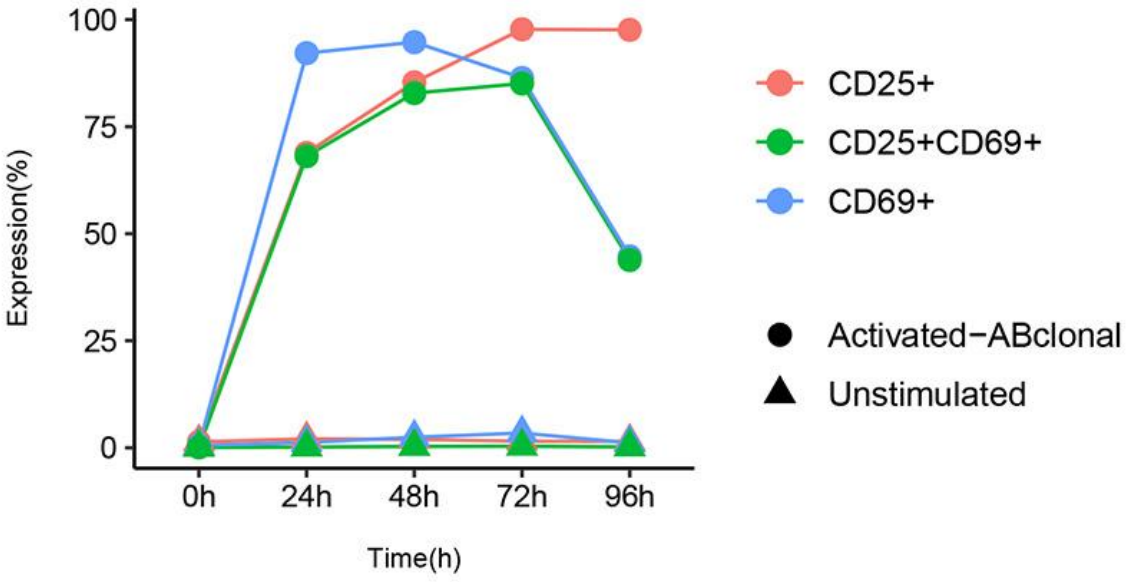
◆ Storage

Store at 2-8℃. 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



C57BL/6 mouse splenocytes were activated using Armenian Hamster anti-Mouse CD3 mAb (A27913,2 μg/ml) and Syrian Hamster anti-Mouse CD28 mAb (A27914,2 μg/ml), or competitor's CD3/CD28 antibody for 72 hours. The unstimulated control was performed without treatment. Cells were fluorescently stained with FITC Anti-Mouse CD3e mAb (A23322,5 μL/Test), ABflo® 647 Rabbit anti-Mouse CD25 mAb (A23808,5 μL/Test), ABflo® 450 Rabbit anti-Mouse CD69 mAb (A26449,5 μL/Test). The degree of T cell activation are defined as $CD25^{+}CD69^{+}$ cells on $CD3^{+}$ T cells gate.



The expression of CD25 and CD69 in mouse $CD3^{+}$ T Cells changes with activation time using Mouse T Cell Activation Kit (Anti-CD3/CD28) (RK40101,2 μg/ml).