

## T Cell Activation with anti-CD3 Antibodies

### Human T Cell Activation with anti-CD3 (clone UCHT1 or HIT3a)

#### Materials:

- Sterile PBS
- Anti-human CD3, clone UCHT1 (LEAF™ format, Cat. No. 300413/4) or clone HIT3a (LEAF™ format, Cat. No. 300313/4)
- Cell culture medium (e.g., RPMI-1640 or IMDM supplemented with 10% FBS and 2mM L-glutamine)
- Sterile single-cell suspension of Ficoll-Hypaque-purified peripheral blood mononuclear cells, isolated T cells, or T cell subsets
- 96-well flat-bottom tissue culture plates with lids (e.g., Costar® Cat. No. 3596)

#### Method:

1. Prepare a 10 µg/ml solution of anti-CD3 (clone UCHT1 or HIT3a) in sterile PBS.
2. Dispense 50 µl of the antibody solution to each microwell of the 96-well assay plate. For the unstimulated control wells, add 50 µl of sterile PBS.
3. Seal plate. Incubate at 37°C for 2 hours or 4°C overnight.
4. Aseptically decant antibody solution from the microwell plate.
5. Wash plate microwells 3 times with sterile PBS. Discard liquid.
6. Prepare single cell suspension of cells of interest in supplemented cell culture medium to 1-2 x 10<sup>6</sup>/ml.
7. Aliquot 200 µl cell suspension into plate microwells. Cover with lid. Incubate at 37°C in 5% CO<sub>2</sub> and 100% humidity for 3 days.

\* Soluble forms of LEAF™ purified UCHT1 (1 µg/ml) or LEAF™ purified HIT3a (0.01 – 0.1 µg/ml) may be used to activate T cells from PBMC cell populations.

## **Mouse T Cell Activation with anti-CD3 $\epsilon$ (clone 145-2C11)**

### **Materials:**

- Sterile PBS
- Anti-mouse CD3 $\epsilon$ , clone 145-2C11 (LEAF™ format, Cat. No. 100313/4)
- Cell culture medium (e.g., RPMI-1640 or IMDM supplemented with 10% FBS and 2mM L-glutamine)
- Sterile, single-cell suspension (e.g., splenocytes, lymph node cells), isolated T cells or T cell subsets
- 96-well flat-bottom tissue culture plates with lids (e.g., Costar® Cat. No. 3596)

### **Method:**

1. Prepare a 5  $\mu\text{g/ml}$  solution of anti-CD3 $\epsilon$  (clone 145-2C11) in sterile PBS.
2. Dispense 50  $\mu\text{l}$  of the antibody solution to each microwell of the 96-well assay plate. For the unstimulated control wells, add 50  $\mu\text{l}$  of sterile PBS.
3. Seal plate. Incubate at 37°C for 2 hours or 4°C overnight.
4. Aseptically decant antibody solution from microwell plate.
5. Wash plate microwells 3 times with sterile PBS. Discard liquid.
6. Prepare single cell suspension of cells of interest.
7. Resuspend cells in supplemented cell culture medium to 1-2 x 10<sup>6</sup>/ml.
8. Aliquot 200  $\mu\text{l}$  cell suspension into plate microwells. Cover with lid. Incubate at 37°C in 5% CO<sub>2</sub> and 100% humidity for 3 days.