BioLegend
The path to legendary discovery™

T Cell Activation with anti-CD3 Antibodies

Human T Cell Activation with anti-CD3 (clone UCHT1, OKT3 or HIT3a)
Materials:

- Sterile PBS
- Anti-human CD3 Antibody
  - Clone UCHT1 (LEAF™ format, Cat. No. 300413/300414/300432; Ultra-LEAF™ format, Cat. No. 300437/300438)
  - Clone OKT3 (LEAF™ format, Cat. No. 317303/317304/317315; Ultra-LEAF™ format, Cat. No. 317325/317326)
  - Clone HIT3a (LEAF™ format, Cat. No. 300313/300314; Ultra-LEAF™ format, Cat. No. 300331/300332)
- 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, OKT3, 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 10^6/ml.
7. Aliquot 200 µl cell suspension into plate microwells. Cover with lid. Incubate at 37°C in 5% CO_2 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-CD3ε (clone 145-2C11)
Materials:

- Sterile PBS
- Anti-mouse CD3ε, clone 145-2C11 (LEAF™ format, Cat. No. 100313/100314; Ultra-LEAF™ format, Cat. No. 100339/100340)
- 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 µg/ml solution of anti-CD3ε (clone 145-2C11) 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 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 10^6/ml.
8. Aliquot 200 µl cell suspension into plate microwells. Cover with lid. Incubate at 37°C in 5% CO2 and 100% humidity for 3 days.