



# T Cell Activation with anti-CD3 Antibodies Protocol - Human V.4

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## Guidelines

*What if I want to stimulate my cells to a particular T-cell lineage?*

- As a guide for this, you can take a look at our [Activation Bundles page](#), which will provide you with the listing of our products you will need to differentiate into different T-helper cell lineages.



## Materials

### MATERIALS

⊗ Anti-human CD3 Antibody: Clone UCHT1 (Ultra-LEAF™ format) **BioLegend Catalog #300437**

⊗ Anti-human CD3 Antibody: Clone HIT3a (Ultra-LEAF™ format) **BioLegend Catalog #300331**

### STEP MATERIALS

⊗ 96-well flat-bottom tissue culture plates with lids **Corning Catalog #3596**

- Sterile PBS
- 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)
- **Note:** Soluble forms of Ultra-LEAF™ purified UCHT1 (1µg/ml) or Ultra-LEAF™ purified HIT3a (0.01 - 0.1µg/ml) may be used to activate T cells from PBMC cell populations.

## Protocol materials

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- 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.

 96-well flat-bottom tissue culture plates with lids **Corning Catalog #3596**
- 3 Seal plate. Incubate at 37°C for 2 hours or 4°C overnight.

 02:00:00
- 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  $1-2 \times 10^6$ /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.