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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Troubleshooting

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 × 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.