



T Cell Activation with anti-CD3 Antibodies Protocol - Human V.3

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Kelsey Knight¹

¹BioLegend

BioLegend

Tech. support email: tech@biolegend.com



Sam Li

BioLegend

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Materials

STEP MATERIALS


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

- 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)

* 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.

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.

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- 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 $1-2 \times 10^6/\text{ml}$.
- 7 Aliquot 200 µl cell suspension into plate microwells. Cover with lid. Incubate at 37°C in 5% CO₂ and 100% humidity for 3 days.