T Cell Activation with anti-CD3 Antibodies Protocol - Human

Kelsey Knight[1]
[1]BioLegend

Sep 13, 2018 dx.doi.org/10.17504/protocols.io.tkbeksn
BioLegend

Version 3
Working

Kelsey Knight
BioLegend

EXTERNAL LINK

https://www.biolegend.com/protocols/t-cell-activation-with-anti-cd3-antibodies-protocol-human/4271/

PROTOCOL STATUS

Working

MATERIALS TEXT

• 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.

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.

REAGENT
96-well flat-bottom tissue culture plates with lids
by 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 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.