Human T Cell Activation with anti-CD3 Anitbodies (clone UCHT1, OKT3 or HIT3a) Version 2

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Abstract

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Guidelines

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)

* 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

Step 1.
Prepare a 10 µg/ml solution of anti-CD3 (clone UCHT1, OKT3, or HIT3a) in sterile PBS.

Step 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.

REAGENTS
96-well flat-bottom tissue culture plates with lids 3596 by Corning

Step 3.
Seal plate. Incubate at 37°C for 2 hours or 4°C overnight.

DURATION

02:00:00

Step 4.

Aseptically decant antibody solution from the microwell plate.

Step 5.

Wash plate microwells 3 times with sterile PBS (wash 1/3).

Step 6.

Wash plate microwells 3 times with sterile PBS (wash 2/3).

Step 7.

Wash plate microwells 3 times with sterile PBS (wash 3/3). Discard liquid.

Step 8.

Prepare single cell suspension of cells of interest in supplemented cell culture medium to 1-2 x $10^{6/}$ml.

Step 9.

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.