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

DOI
dx.doi.org/10.17504/protocols.io.794hr8w

Sam Li[1]

[1]BioLegend

BioLegend
Tech. support email: **tech@biolegend.com**

Sam Li
BioLegend

DOI: dx.doi.org/10.17504/protocols.io.794hr8w

External link: https://www.biolegend.com/protocols/t-cell-activation-with-anti-cd3-antibodies-protocol-mouse/4245/

Protocol Citation: Sam Li . T Cell Activation with anti-CD3 Antibodies Protocol - Mouse. **protocols.io https://dx.doi.org/10.17504/protocols.io.794hr8w**

Created: October 16, 2019

Last Modified: October 16, 2019

Protocol Integer ID: 28700

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-mouse CD3ε clone 145-2C11 (Ultra-LEAF™ format) **BioLegend Catalog #**100339

- Sterile, single-cell suspension (e.g., splenocytes, lymph node cells), isolated T cells or T cell subsets
- 96-well flat-bottom tissue culture plates with lids (e.g., Costar® Cat. No. 3596)
- Cell culture medium (e.g., RPMI-1640 or IMDM supplemented with 10% FBS and 2mM L-glutamine)
- Sterile PBS

1 Prepare a 5µg/ml solution of anti-CD3ε (clone 145-2C11) in sterile PBS.

2 Dispense 50µl of the antibody solution to each microwell of the 96-well assay plate. For the unstimulated control wells, add 50µl of sterile PBS.

3 Seal plate. Incubate at 37°C for 2 hours or 4°C overnight. 02:00:00

4 Aseptically decant antibody solution from microwell plate.

5 Wash plate microwells 3 times with sterile PBS. Discard liquid.

6 Prepare single cell suspension of cells of interest.

7 Resuspend cells in supplemented cell culture medium to 1-2 × 10^6/ml.

8 Aliquot 200µl cell suspension into plate microwells. Cover with lid. Incubate at 37°C in 5% CO^2 and 100% humidity for 3 days.