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

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[1]BioLegend

1 Works for me dx.doi.org/10.17504/protocols.io.794hr8w

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EXTERNAL LINK

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

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

NAME	CATALOG #	VENDOR
Anti-mouse CD3ε clone 145-2C11 (Ultra-LEAF™ format)	100339	BioLegend

MATERIALS TEXT

- 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.

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7 Resuspend cells in supplemented cell culture medium to 1-2 x 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.

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