



T Cell Activation with anti-CD3 Antibodies Protocol - Mouse [↗](#)

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¹BioLegend

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Version 2

BioLegend

Working



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EXTERNAL LINK

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

PROTOCOL STATUS

Working

MATERIALS TEXT

- Sterile PBS
- Anti-mouse CD3 ϵ , clone 145-2C11 (LEAF[™] format, Cat. No. 100313/100314; Ultra-LEAF[™] format, Cat. No. 100339/100340)
- Cell culture medium (e.g., RPMI-1640 or IMDM supplemented with 10% FBS and 2mM L-glutamine)
- 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)

- 1 Prepare a 5 μ g/ml solution of anti-CD3 ϵ (clone 145-2C11) in sterile PBS.
- 2 Dispense 50 μ l of the antibody solution to each microwell of the 96-well assay plate. For the unstimulated control wells, add 50 μ l 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 x 10⁶/ml.

8 Aliquot 200µl cell suspension into plate microwells. Cover with lid. Incubate at 37°C in 5% CO² and 100% humidity for 3 days.



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