



T Cell Activation with anti-CD3 Antibodies Protocol - Mouse V.2

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Materials

- 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 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 $1-2 \times 10^6$ /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.