

Mouse T Cell Activation with anti-CD3 ϵ Antibodies (clone 145-2C11)

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Abstract

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Guidelines

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)

Protocol

Step 1.

Prepare a 5 μ g/ml solution of anti-CD3 ϵ (clone 145-2C11) in sterile PBS.

Step 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.



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 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.

Step 9.

Resuspend cells in supplemented cell culture medium to $1-2 \times 10^6/\text{ml}$.

Step 10.

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

 DURATION

12:00:00