

June 2013

CD3 Plate Stimulation Protocol of Murine T Cells for the Detection of Activation Markers

Reagents and Materials

Full Name	Short Name	Catalog Number
Sterile 1xPBS		
CD3e, Clone 145-2C11, NA/LE anti-mouse	CD3e	553057
CD28, Clone 37.51, NA/LE anti-mouse (optional)	CD28	553294
96-well OR 6-well flat-bottom plates	flat-bottom plates	

Procedural Note

- Determine a precise incubation time by performing a time course experiment and assay for your activation marker.

Procedure, CD3 only

- Coat a dish, flask, or plate with 10 µg/mL of anti-mouse CD3 in sterile PBS and incubate overnight at 4°C.
- Wash three times with 10 mL of sterile PBS to remove non-bound soluble antibody.
- Add 2×10^6 splenocytes/mL and incubate 1 to 4 days at 37°C.

Procedure, co-stimulation using CD3 and CD28

- Coat flat-bottom plates with 200 µL per well (96-well plate) or 2 mL per well (6-well plate) of CD3 at 10 µg/mL concentration and incubate overnight at 4°C.
- Wash three times with 10 mL of sterile PBS.
- Dispense $1-2 \times 10^6$ cells per well (for a 96-well plate) or $4-6 \times 10^6$ cells per well for 6-well plate.
- Add CD28 (2 µg/mL) to wells.
- Incubate 1 to 4 days at 37°C.



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