Protocol for CD3 Stimulation of Human T Cells for the Detection of Activation Markers

Materials and Reagents

Full Name	Short Name	Catalog Number
Ficoll-Paque™ Plus	Ficoll-Paque Plus	
Suspension medium supplemented with 1% glutamine, 1% penicillin/streptomycin, and 10% FBS	medium	
CD3, clone HIT3a, soluble NA/LE format (for soluble option)	CD3 clone HIT3a	555336
CD3, clone UCHT1 (for immobilized option)	CD3 clone UCHT1	555329
BD Falcon™ 96-well U-bottom microtiter plate, or equivalent	round-bottom plate	353918
CD28, clone CD28.2, soluble NA/LE format (for co-stimulation option)	CD28	555725
Protein G (Sigma) (for co-stimulation enhancement)	Protein G	

Procedural Notes

- Select one CD3 clone according to the format you want to use. Clone HIT3a is optimal for the soluble format, while clone UCHT1 is optimal for the immobilized format.
- To use the immobilized format, coat the CD3 UCHT1 antibody onto a plate at 5 to 10 µg/mL at 4°C, overnight.
- Either clone can be used in combination with CD28 for co-stimulation.
- Addition of Protein G significantly enhances co-stimulation, presumably through cross-linking.

Procedure

1. Isolate human PBMCs with Ficoll-Paque Plus according to the manufacturer's instructions.
2. Suspend PBMCs in medium at 10^6 cells/mL.
3. Do one of the following depending on the format you are using:
   - For the soluble format, incubate 10^6 cells with CD3 clone HIT3a at 1 µg/mL final concentration in culture for 3 days (7% CO_2, 37°C).
   - For the immobilized format, add cells to the CD3 clone UCHT1-coated plate.
4. Wash the cells twice and resuspend them in medium at 10^6 cells/mL.
5. Distribute the cells in a round-bottom plate at 10^5 cells per well.
6. [Optional for co-stimulation] Add CD28, clone CD28.2 and optionally Protein G at 5 µg/mL each final concentration, and incubate the plate for an additional 3 days (5–7% CO_2 at 37°C).