Unprimed T Cell Activation – Antibody Stimulation Methods

Protocol

FC16

This method provides a general procedure for activating T cells prior to staining for intracellular cytokines, activation markers or cell proliferation using anti-CD3 and anti-CD28 antibodies, see Table 1. Blood samples must be collected in heparin anticoagulant as EDTA will interfere with the cell stimulation process.

This protocol is for use with the majority of Bio-Rad reagents. In some cases specific recommendations are provided on product datasheets, and these methods should always be used in conjunction with the product and batch specific information provided with each vial. A certain level of technical skill and immunological knowledge is required for the successful design and implementation of these techniques; these are guidelines only and may need to be adjusted for particular applications.

Reagents

- Phosphate buffered saline (PBS, cat. #BUF036A)-CD3 and CD28 antibodies - see Table 1
- Anti-CD3 and CD28 antibodies - see Table 1
- Cell media

Table 1. Anti-CD3 and CD28 Antibodies

Antibody	Clone	Cells	Catalog #
Mouse Anti-Human CD3	UCHT1	Human	MCA463EL
Rat Anti-Human CD28	YTH913.12	Human	MCA709EL
Hamster Anti-Mouse CD3	145-2C11	Mouse	MCA2690
Mouse Anti-Mouse CD28: LE	E18	Mouse	MCA2473EL

Method

1. Prepare a 5-10 µg/ml solution of anti-CD3 in sterile PBS.
2. Add 50–100 µl to each well of a 96 well plate, seal and incubate overnight at 4°C or 1–2 hr at 37°C. For the unstimulated control add 50–100 µl of sterile PBS.
3. Remove supernatant and wash the plate with PBS to remove unbound antibody.
4. Isolate mononuclear cells using the appropriate methods, for example, for human peripheral blood refer to protocol FC2 Preparation of Human Peripheral Blood Mononuclear Cells, for mouse spleen refer to protocol FC3 Preparation of Peritoneal Macrophages, Bone Marrow, Thymus and Spleen Cells.
5. Resuspend in the appropriate media and adjust to 1 x 10^6 cells/ml.

Optional step:
For proliferation studies using CytoTrack™ Cell Proliferation Assay or CFSE, incubate cells with the dye following the recommended protocol, or see protocol FC18 Measuring Cell Proliferation Using Cell Permeable Dyes.

6. Add 1 x 10^5 cells to each well.
7. Add CD28 antibody at 1–5 µg/ml and incubate cells in a humidified 37°C, 5% $C0_2$ incubator for 6–96 hrs as required.
8. Harvest cells and perform surface or intracellular staining as required. Note: cytokine staining requires golgi inhibitors such as brefeldin A and monensin, see protocol FC9 Direct Immunofluoresence Staining of Intracellular Cytokines in Blood for more details.
9. Acquire by flow cytometry.

Notes

Appropriate controls should be carried out for flow cytometry, consider including the following:

- Unstained cells (should always be included to monitor autofluorescence)
- Unstimulated control

Visit **bio-rad-antibodies.com/applications** for more information about flow cytometry.

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