BIO-RAD

Primed T-Cell Activation: Antigen-Presenting Cell Co-Culture Method

FC17

This method provides a general procedure for reactivating antigen-experienced (primed or memory) T-cells prior to staining for intracellular cytokines, activation markers, or cell proliferation. T cells are co-cultured with an antigen-presenting cell, in this case, dendritic cells that have been pulsed with an antigen of interest.

This protocol provides a general staining procedure for use with Bio-Rad reagents. Specific recommendations are provided on product datasheets, and those instructions should always be used in conjunction with the product- and batch-specific information provided with each antibody vial. A certain level of technical skill is required for the successful design and implementation of these techniques; these are guidelines only and may need to be adjusted for particular cell types or applications.

Reagents:

- Cell culture medium
- Dendritic cells from an appropriate source, such as murine bone marrow cells cultured in 10 mg/mL GM-CSF
- Antigen of interest, such as a synthetic peptide
- Lipopolysaccharide (LPS)
- Required when measuring proliferation:
  - CytoTrack Cell Proliferation Assay Kit (catalog #1351202, 1351203, or 1351205) or CFDA-SE Cell Proliferation Assay Kit (#1351201)
- Phosphate buffered saline, 10x (PBS) (#BUF036A)
- Required when staining cell surface antigens:
  - Staining Buffer (#BUF073)

Method:

1. Pulse dendritic cells with antigen (50–200 µg/mL) by incubating overnight in the presence of LPS (100 ng/mL). Antigen should be titrated to obtain reproducible results.
Note: Non-pulsed dendritic cells should be included as a negative control.
2. Wash the dendritic cells twice in cell culture media. Resuspend cells in cell culture media and adjust to 1 x 10^6 cells/mL.
3. Isolate T cells and resuspend in media at 1 x 10^6 cells/mL.
4. **Optional:** For proliferation studies using CytoTrack Cell Proliferation Assay or CFDA-SE Cell Proliferation Assay, incubate cells with the dye following the recommended protocol, or see protocol **FC18**, Measuring Cell Proliferation Using CytoTrack, a Cell-Permeable Dye.
5. Co-culture the dendritic cells and T cells at increasing ratios, for example, 1:1, 1:5, and 1:10 (DC:T cells) to obtain a range of stimulation.
6. Incubate cells in a humidified 37°C, 5% CO_2 incubator for 6–96 hr as required.
7. Centrifuge at 300–400 x g for 5 min and discard supernatant.
8. Perform staining of cell surface antigens (protocol **FC4**, Direct Immunofluorescence Staining of Surface Epitopes of Cells and Whole Blood) or intracellular staining (protocol **FC7**, Direct Immunofluorescence Staining of Intracellular Antigens: The Leucoperm Method) as required along with associated wash steps.
Note: Cytokine staining requires protein transport inhibitors such as brefeldin A and monensin. See protocol **FC9**, Direct Immunofluoresence Staining of Intracellular Cytokines in Whole Blood, for more details.
9. Acquire samples on a flow cytometer.

Notes:

- Other types of antigen-presenting cells may be used but could require pulsing with different amounts of antigen and different incubation times
- All antibodies should be titrated prior to use to ensure that the optimal concentration is used
- Appropriate controls should be included; for example, an unstimulated control and incubation with non-pulsed dendritic cells

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