Intracellular Cytokine (INF-gamma) Staining Assay

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[Abstract] An intracellular cytokine (INF-gamma) staining assay is used to analyze the function of lymphocytes at the single cell level. By combining surface staining and intracellular cytokine staining, this assay can reveal the percentage of cytokine-releasing cells in a particular population, which cannot be obtained from an ELISpot assay.

Materials and Reagents

1. PE Rat Anti-Mouse IFN-γ (BD Biosciences, catalog number: 554412)
2. PE Rat IgG1 κ Isotype Control (BD Biosciences, catalog number: 554685)
   Note: The above antibodies have been tested by the author and may be substituted with the antibodies desired by users.
3. BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit with BD GolgiStop™ (BD Biosciences, catalog number: 554715)
4. 1x phosphate buffered saline (PBS)
5. FACS staining buffer (1x PBS, 2% FBS)
6. Phorbol 12-myristate 13-acetate (PMA) (Sigma-Aldrich, catalog number: 79346)
7. Ionomycin (Sigma-Aldrich, catalog number: I9657)

Equipment

1. BECKMAN centrifuges and rotor (Beckman Coulter)
2. Incubator
3. 96 well plates
4. Falcon round-bottom tubes

Procedure

A. Stimulation of cells

1. For stimulating IFN-gamma release from T cells, add PMA (5 ng/ml) and ionomycin (500 ng/ml) to the cell culture and incubate for 6 h at 37 °C.

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2. Add 4 µl of BD GolgiStop™ for every 6 ml of cell culture and mix thoroughly (it is recommended that BD GolgiStop not be kept in cell culture for longer than 12 h).

B. Harvest cells

3. Collect the cells and centrifuge at 1,500 rpm for 3 min.
4. Wash twice with PBS.
5. Dilute single-cell suspensions to 1 x 10^7 cells/ml in PBS with 2% FBS.
6. Add 100 µl cells (1 x 10^6) per well in 96 well plates.
7. Spin plate at 800 *x g*, 3 min, at 4 °C.
8. Wash 3 times with cold PBS, spinning as in step 7.

C. Stain cell surface antigens

9. Resuspend the cells in 100 µl Fc block (recommended dilution: 1:1,000 in PBS/2% FBS). Incubate on ice, 10 min. Spin.
10. Resuspend in 100 µl surface antibody mixture (recommend dilution: 1:100 in PBS/2% FBS). Incubate at room temperature, 20 min in the dark. Spin.
11. Wash once with cold PBS.

D. Stain intracellular antigens

12. Resuspend in 200 µl of BD Cytofix/CytoPerm solution. Incubate at room temperature, 30 min in the dark. Spin 1500 *x g*, 3 min, 4 °C.
13. Wash twice with 200 µl BD Perm/wash buffer. Spin as in step 12.
14. Resuspend in 100 µl cytokine stain (recommended dilution: 1:100 in 1x Perm/Wash). Incubate on ice, 30 min in the dark. Spin as in step 12.
15. Wash twice with BD Perm/Wash, spinning as in step 12.
16. Resuspend cells in 300-400 µl FACS buffer and transfer to Falcon round-bottom tubes for acquisition on a flow cytometer.

Acknowledgments

This protocol was previously used in Assenmacher *et al.* (1994) and Shang *et al.* (2004).

References

1. Assenmacher, M., Schmitz, J. and Radbruch, A. (1994). Flow cytometric determination of cytokines in activated murine T helper lymphocytes: expression of interleukin-10 in interferon-gamma and in interleukin-4-expressing cells. *Eur J Immunol* 24(5): 1097-1101.

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