

ANTIGEN-SPECIFIC INDUCIBLE CYTOKINE ASSAY

1. INTRODUCTION

Cytokines are crucial components of the immune response. T helper-derived cytokines including IL2 and IFN γ shift the immune response towards cell-mediated processes (type 1), whereas IL4, IL5, and IL10 promote antibody production (type 2). The type and amount of cytokines produced during antigen stimulation contribute important information regarding the immune response to specific antigens.

The antigen-specific inducible cytokine production measures the amount of cytokine secreted in the supernatant of antigen-stimulated peripheral blood mononuclear (PBMC) cultures. The assay consists of PBMC stimulation, supernatant harvesting and cytokine measurement using commercial EIA kits. The kinetics of inducible cytokines significantly varies with the type of cytokine but not with the stimulating antigen. For IL2 and IL10, peak cytokine secretion occurs on the 3rd day of antigen-specific PBMC stimulation, whereas IFN γ reaches peak stimulation between days 5 and 7.

2. REAGENTS

- 5×10^6 PBMC
- RPMI 1640 with glutamine
- Heat-inactivated human AB serum
- Penicillin/ streptomycin antibiotic solution
- CMV or VZV antigen and control
- 96-well round-bottom plates
- Cytokine-specific EIA kits (recommended manufacturers : Endogen for IL2 and IFN γ and Immunotech for IL10)

3. ASSAY PROCEDURE

3.1. Preparation of Plates (one to several plates can be prepared at one time)

- 3.1.1. Calculate the volume of medium that needs to be prepared: $0.1\text{ml/well} \times n^{\circ}$ of antigens (e.g. a microbial antigen and an appropriate control) $\times n^{\circ}$ of cytokines to be measured $\times n^{\circ}$ of patients. Please note that only one well is necessary for each cytokine/antigen pair.
- 3.1.2. Prepare an adequate volume of 2X growth medium (2X GM) represented by RPMI 1640 with glutamine containing 20% human AB serum and 2% antibiotics.
- 3.1.3. Make antigen dilutions in 2X GM at pre-established optimal concentration of antigen (this will vary with the antigen batch).
- 3.1.4. On individual patient plates, designate and label wells for each antigen.
- 3.1.5. Dispense 0.1 ml of antigen in the pre-determined wells.
- 3.1.6. Use plates or store them at -20°C or lower.

3.2. Assay Set-up and Harvest

- 3.2.1. Make or thaw antigen plates.
- 3.2.2. Using PBMC separated with ficoll-hypaque gradients or CPT tubes (washed and counted) make a 4×10^6 cells/ml suspension by diluting 4×10^6 cells in 1 ml of RPMI.
- 3.2.3. Add 0.1 ml of the 4×10^6 cells/ml suspension to each antigen well (one antigen well/ cytokine).
- 3.2.4. Incubate plates at 37°C in a 5% CO_2 , 90% humidified atmosphere for the following n° of days:
 - 3.2.5. 3 days for IL2 and/or IL10
 - 3.2.6. 6 days for $\text{IFN}\gamma$
- 3.2.7. On the designated harvest day, with a micropipette, aspirate 150 μl of supernatant, carefully avoiding the cells at the bottom of the well.
- 3.2.8. Save the supernatant in pre-labeled cryovials at 4°C , if the EIA assay is scheduled within 24 h, or at -70°C , for longer time intervals.

3.3. EIA Assay and Calculations

As per manufacturer's instructions.

Example of a plate set-up for one patient, CMV antigen, 3 cytokines

	CMV IL2		Control IL2*								
	CMV IFN		Control IFN*								
	CMV IL10		Control IL10								

*Optional