Human IFN-γ Matched Antibody Pair for ELISpot

Pre-titered capture antibody and biotinylated detection antibody matched pair for the development of enzyme-linked immunospot (ELISpot) assays for the quantitation of single cells releasing human interferon-γ (IFN-γ).

Catalogue Number: SL10024A

Designed for 5 x 96 tests

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTENDED USE

Pre-titered capture antibody and biotinylated detection antibody matched pair for the development of enzyme-linked immunospot (ELISpot) assays for the quantitation of single cells releasing human IFN-γ.

A recommended assay protocol is provided. The dilutions of capture antibody and detection antibody is determined according to this protocol. The researcher can optimize the dilutions if it is necessary.

For laboratory research use only. Not for use in diagnostic procedures.

INTRODUCTION

Interferon gamma (IFN-γ) is a multifunctional protein first observed to have antiviral activity in cultures of Sindbis virus-infected human leukocytes stimulated by PHA. [(1))] The biochemistry and biological activities of the interferons have been extensively reviewed. Produced by both $CD4^+$ and $CD8^+$ T lymphocytes and natural killer (NK) cells, INF-γ is now known to be both an inhibitor of viral replication and a regulator of numerous immunological functions. IFN-γ influences the class of antibody produced by B cells up-regulates classes I and II MHC complex antigens and increases the efficiency of macrophage-mediated killing of intracellular parasites**.** [(2, 3)] Most of the activities attributed to IFN-γ are believed to be mediated by IFN-γ-induced proteins. The appearance of such proteins is a consequence of IFN-γ binding to a specific receptor that is distinct from the receptor for IFN-α and β. [(4)] Human IFN-γ is reported to be active only on human and non-human primate cells. [(5)] The biochemistry and biological activities of the interferons have been extensively reviewed. [(2-9)]

Human IFN-γ is a 143 amino acid residue glycoprotein with MW of 20 or 25 kDa that demonstrates little sequence homology to IFN-α and β. [(10-13)] Naturally occurring IFN-γ is found as either of two molecular-weight-species, differing in degree of glycosylation. The 25 kDa species is glycosylated at both potential N-linked glycosylation sites on the molecule (Asn 25 and 97), while the 20 kDa species is glycosylated only at Asn97. [(17, 18)] In neither case glycosylation is required for biological activity. [(13, 16)] Two allelic variants of IFN-γ have been described differing by the presence of an Arg or Gln at position 137. [(10, 16)]

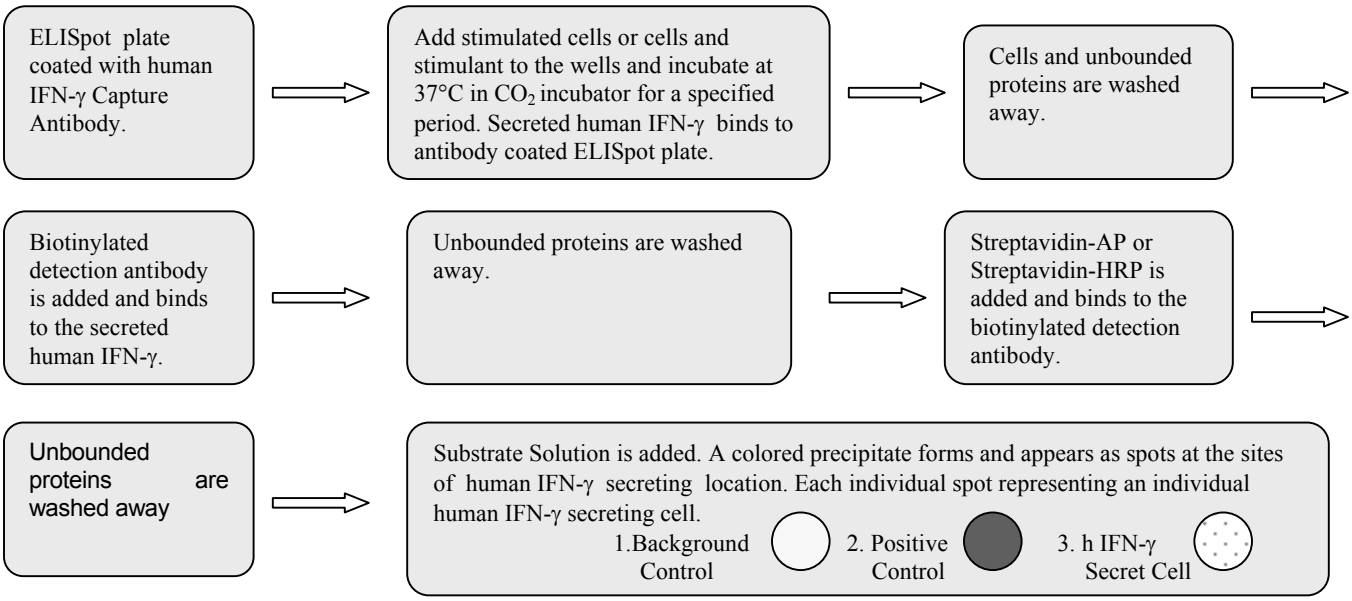
Although the cDNA encoding for IFN-γ predicts a protein of 146 amino acid residues, the form secreted by mammalian cells shows a truncation of three amino acid residues from the N-terminus and the conversion of the fourth residue from glutamic acid to pyroglutamate. [(11)] The secreted form of IFN-γ has no potential for the formation of disulfide bonds. [(13)] Human IFN-γ apparently exists as a head-to-tail dimmer in solution with the C-terminus of one monomer aligned with the N-terminus of the other monomer. [(18, 19)]

IFN-γ possesses a variety of functions. Produced by CD8+, NK and TH2 T helper cells, IFN-γ has documented antiviral, antiprotozoal and immunomodulatory activities, [(20-24)] although IFN-α and IFN-β seem to have more potential antiviral activities than IFN-γ. [(24)] The antiprotozoal activity of IFN-γ against Toxoplasma and Chlamydia is believed to result from indoleamine 2, 3-dioxygenase activity,

an enzyme induced by IFN-γ. [25] The immunomodulatory effects of IFN-γ are extensive and diverse. In monocyte/ macrophages, IFN-γ increases expression of class 1 MHC antigens; increases the production of IL-1, platelet-activating factor, H_2O_2, and pterin; protects monocytes against LAK cell-mediated lysis; down-regulates IL-8 mRNA expression that is up-regulating TGF-β receptor expression and up-regulating expression of the IL-2Rγ subunit. [23, 25, 26-29] It has also been demonstrated to be chemotactic for monocytes but not neutrophils. [30] IFN-γ selectively enhances both Ig G_{2a} secretion by LPS-stimulated B cell activation. [31, 32] IFN-γ has also been reported to induce its own expression. IFN-γ production accompanying local inflammation results in the induction of IFN-γ mRNA synthesis at distant sites. This effect could be due to circulating IFN-γ or the production of IFN-γ by migrating cells [33]. IFN-γ has also been shown to up-regulate ICAM-1 but not E-selectin or VCAM-1 expression on endothelial cells [34]. Finally, IFN-γ has recently been implicated in the development of a cholinergic phenotype in embryonic septal neurons. In cultures of rat septal nuclei, IFN-γ induced the development of cholinergic neurons.

This ELISpot kit is developed to detect and visualize of single cells secreting human IFN-γ.

PRINCIPLES OF THE ASSAY



REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

Name (Part No.)	Size	Description	Usage and Storage
1)Concentrated human IFN-γ Capture Antibody (Part SL10024A-1)	1 Vial	Lyophilized mouse anti-human IFN-γ monoclonal antibody	Stock Solution: Reconstitute Concentrated Human IFN-γ Capture Antibody with 0.6 mL PBS. Aliquot if repeated use is expected. The stock solution can be stored frozen (-20˚C to -70˚C) for up to 6 months. Avoid freeze-thaw cycles. Working Solution: When PVDF -bottom Immunospot plates are used, the recommended dilution is 1: 100. Calculate the volume of Capture Antibody Stock Solution needed and dilute to working solution in PBS. Use in 1 hour.
2)Concentrated human IFN-γ Detection Antibody (Part SL 10024A-2)	1 Vial	Concentrated Biotinylated mouse anti-human IFN-γ monoclonal antibody	Stock Solution: Reconstitute Concentrated human IFN-γ Detection Antibody with 0.6 mL Reagent Diluent. Aliquot if repeated use is expected. The stock solution can be stored frozen (-20˚C to -70˚C) for up to 6 months. Avoid freeze-thaw cycles. Working Solution: When PVDF -bottom Immunospot plates are used, the recommended dilution is 1: 100. Calculate the volume of Detection Antibody Stock Solution needed and dilute to working solution in Reagent Diluent. Use in 1 hour.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. **PBS**
   137mM NaCl, 2.7mM KCl, 8.1mM Na_2HPO_4, 1.5mM KH_2PO_4, pH 7.2-7.4, 0.2μm filtered.
2. **Wash Buffer**
   0.05% Tween-20 in PBS.
3. **Blocking Buffer**
   1% BSA, 5% Sucrose in PBS, 0.2μm filtered.
4. **Reagent Diluent**
   1% BSA in PBS.
5. **Positive Control (Recommended)**
   Recombinant human IFN-γ (2ng/vial, Catalogue Number SL10024C) or equivalent.
6. **ELISpot Plates**
   PVDF -bottom Immunospot plates or equivalent.
7. **Streptavidin-AP or Streptavidin-HRP**
8. **Substrate Solution**
   8.1 Substrate Solution for Streptavidin-AP color system.
   BCIP/NBT Substrate Solution for ELISpot (10mL/bottle, Catalogue Number SS6006) or equivalent for Streptavidin-AP color system.
   8.2 AEC Substrate Solution for Streptavidin-HRP color system.
   8.2.1 0.1M Phosphate-Citrate Buffer (PH5.0)

Citric Acid Solution: 9.6g Citric Acid to 500 mL Deionized or Distilled Water.
Dibasic Sodium Phosphate Solution: 14.2 g Dibasic Sodium Phosphate to 500mL Deionized or Distilled Water.
Add Dibasic Sodium Phosphate Solution to Citric Acid Solution until the pH to 5.0.
1:1 Dilute with Deionized or Distilled Water.

8.2.2 Dissolve 4 mg of AEC (3-amino-9-ethyl-carbazole) in 1 mL of DMF (Dimethyl Formamide).
8.2.3 Add 14 mL of 0.1M Phosphate-Citrate Buffer (PH5.0)
8.2.4 Filter through a 0.45 μm filter.
8.2.5 Just before use, add 10 μL of 30% H_2O_2.

9. **Pipettes with disposable tips, test tubes and racks, graduated cylinders, absorbent paper, and squirt bottle.**
10. **37˚C CO_2 incubator.**
11. **Deionized or Distilled Water.**
12. **Dissection microscope or ELISpot reader.**

PRECAUTIONS

1. Allow kit reagents and materials to reach room temperature (20-25ºC) before use.
2. Do not use kit components beyond their expiration date. Do not substitute reagents from one kit lot to another.
3. The toxicity of the Substrate Solution is not currently known, wear gloves to avoid contact with skin. Follow local, state and federal regulations to dispose of used Substrate Solution.
4. If 20 x Wash Buffer Concentrated is stored at lower temperature (2-8 ºC), crystals may form which must be dissolved by warming prior to use.
5. When samples are added to the wells, don't let the pipette tips contact the membrane.
6. Don't let the plate dry during the assay.
7. In order to avoid edge effect don't stack plates during cell incubation.
8. Avoid move the plate during cells incubation period.
9. Don't dry the plate at a temperature higher than 37° C.
10. Spots can't be counted accurately until PVDF membranes were completely dry.

SAMPLE PREPARATION

Each researcher should optimize cell separation method, stimulant, stimulation mode and incubation time.
A recommended method to stimulate human IFN-γ secretion from peripheral blood mononuclear cells (PBMCs) is as following:

1. Add 5 x 10^5 /mL PBMCs in 50 ng / mL phorbol 12-myristate-13-acetate and 0.5 ug/mL calcium inomycine.
2. Incubate for 12-18 hours at 37° C in CO_2 incubator.
3. Test according to this protocol.

ASSAY PROCEDURE

Aseptic Procedures: *Steps 1 to 7 are aseptic procedures. Use sterile buffers and aseptic conditions, use laminar flow hood for procedures.*

1. Prepare Human IFN-γ Capture Antibody Working Solution
   As described in **REAGENT PROVIDED.**
2. Add 100 µL of Human IFN-γ Capture Antibody Working Solution to each well of the plate. Cover the plate and incubate overnight at 2-8 ˚C.
3. Wash 3 times with PBS
   Decant or aspirate contents of the plate into a waste container. Fill each well completely with PBS then decant or aspirate contents of the plate into a waste container. Repeat this procedure 2 more times for a total of 3 washes. After final wash, invert plate, and dry by tapping plate onto absorbent paper slightly.
4. Blocking
   Immediately add 200 µL of Blocking Buffer to each well of the plate. Cover the plate and incubate 2 hours at 37˚C.
5. Prepare Positive Control
   We recommend adding 2 wells positive control. If IFN-γ Positive Control (2ng/vial, Catalogue Number SL10024C) was used, add 250 µL Cell Culture Media to each vial. The final concentration is 8 ng/mL. Use within one hour of reconstituting. The reconstitution can be stored frozen (-20°C) for up to 30 days.
6. Wash 1 time with Cell Culture Media
   Decant or aspirate contents of the plate into a waste container. Fill each well completely with Cell Culture Media. Don't discard until cells are ready to be plated.
7. Decant or aspirate contents of the plate into a waste container, invert plate, and dry by tapping plate onto absorbent paper slightly. Immediately add 100 µL IFN-γ secreting cells with appropriate concentration to each well. We recommend adding 2 wells positive control, 2 wells negative control (unstimulated cells), and 2 wells background control (sterile cell culture media) in each plate, 100 µL/well. Incubate at 37°C CO_2 incubator for 4-48 hours. Each researcher should determine the optimal incubation time based on the characteristics of the cell.

Non-aseptic Procedures: *The following steps are non-aseptic procedures.*

8. Prepare Human IFN-γ Detection Antibody Working Solution
   As described in **REAGENT PROVIDED.**
9. Prepare Streptavidin - AP or Streptavidin-HRP Working Solution
   Each researcher should optimize the concentration of Streptavidin - AP or Streptavidin-HRP Working Solution. Calculate the volume of Streptavidin - AP or Streptavidin-HRP Stock Solution needed and dilute to working solution in Reagent Diluent. Use in 1 hour.
10. Wash the plate 5 times with Wash Buffer
    Decant or aspirate contents of the plate into a waste container. Fill each well completely with Wash Buffer then decant or aspirate contents of the plate into a waste container. Repeat this procedure 4 more times for a total of 5 washes. After final wash, invert plate, and dry by tapping plate onto absorbent paper slightly.

11. Immediately add 100 µL of Human IFN-γ Detection Antibody Working Solution to each well of the plate. Cover the plate and incubate 1hour at room temperature (20-25 °C).
12. Repeat wash procedure as described in step 10. Wash plate 5 times.
13. Immediately add 100 µL of Streptavidin-AP or Streptavidin-HRP Working Solution to each well of the plate. Cover the plate and incubate 1hour at room temperature (20-25 °C).
14. Repeat wash procedure as described in step 10. Wash plate 5 times.
15. Immediately add 100 µL of Substrate Solution to each well of the plate. Cover the plate and incubate 5-15 minutes at room temperature (20-25 °C) in dark. Each researcher should optimize the incubate time depending on the plate, reagents or substrate solution used.
16. Stop the assay
    Rinse 5 times with deionized water/distilled water. After final wash, invert plate, and dry by tapping plate onto absorbent paper slightly.
17. Dry plate
    Wet plates show higher background than completely dry plates. Remove the plastic underdrain of the plate. Allow the plate dry for 60-90 min at room temperature, or over night at room temperature, or 15-30 min at 37° C in dark. We recommend dry plate over night at room temperature.
18. Quantify spots using a dissection microscope or ELISpot reader.
19. Dried plate can be stored in sealed plastic bag in dark for 6 months.

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