Human IL- 8 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 IL-8.

Catalogue Number: SL10008A

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 IL-8.

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.

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INTRODUCTION

Interleukin-8 (IL-8), also known as neutrophil attractant/activating protein (NAP-1), monocyte-derived neutrophil-activating peptide (MONAP), monocyte-derived neutrophil chemotactic factor (MDNCF), T lymphocyte chemotactic factor (TCF) and leukocyte adhesion inhibitor (LAI), is a member of the chemokine superfamily which selectively chemoattract and activate specific leukocyte subpopulations (1,2). All of these cytokines have four conserved cysteines and two distinguishable subfamilies. These two subfamilies are dependent on the position of the first two cysteines, which are either separated by on amino acid (C-X-C proteins) or are adjacent (CC-protein) to each other. The members of the two subfamilies differ in their target cell selectivity as well as the chromosomal location of their genes (chromosome 4 for the C-X-C proteins and chromosome 17 for the C-C proteins). IL-8 belongs to the C-X-C subfamily along with platelet factor 4 (PF4), platelet basic protein (PBP), connective-tissue-activating peptide III (CTAPIII), β-thromboglobulin, neutrophil-activating peptide-2 (NAP-2), ENA-78 (3), three closely related MGSA/CRO gene products (GRO-α, GRO-β, GRO-γ), and γ-interferon-inducible protein (γ-IP-10)(4). The members of the C-C chemokines are mainly chemotactic for monocytes whereas the C-X-C chemokines except for IP10 and PF4, chemoattract and activate neutrophils. In addition to the effect on neutrophils, IL-8 has been reported to be a less potent chemoattractant for T lymphocytes (5).

IL-8 is produced by many cells in response to inflammatory stimuli such as IL-1β or TNF-α and to various types of mitogen, lectins, crystals, viruses, and phorbol esters (PMA). Many cell types that produce IL-8 in response to these stimuli can include: monocytes/ macrophages, T lymphocytes, neutrophils, fibroblasts, keratinocytes, hepacytes, chondrocytes, endothelial cells, glioblatoma cells , and mesothelial cells (6).

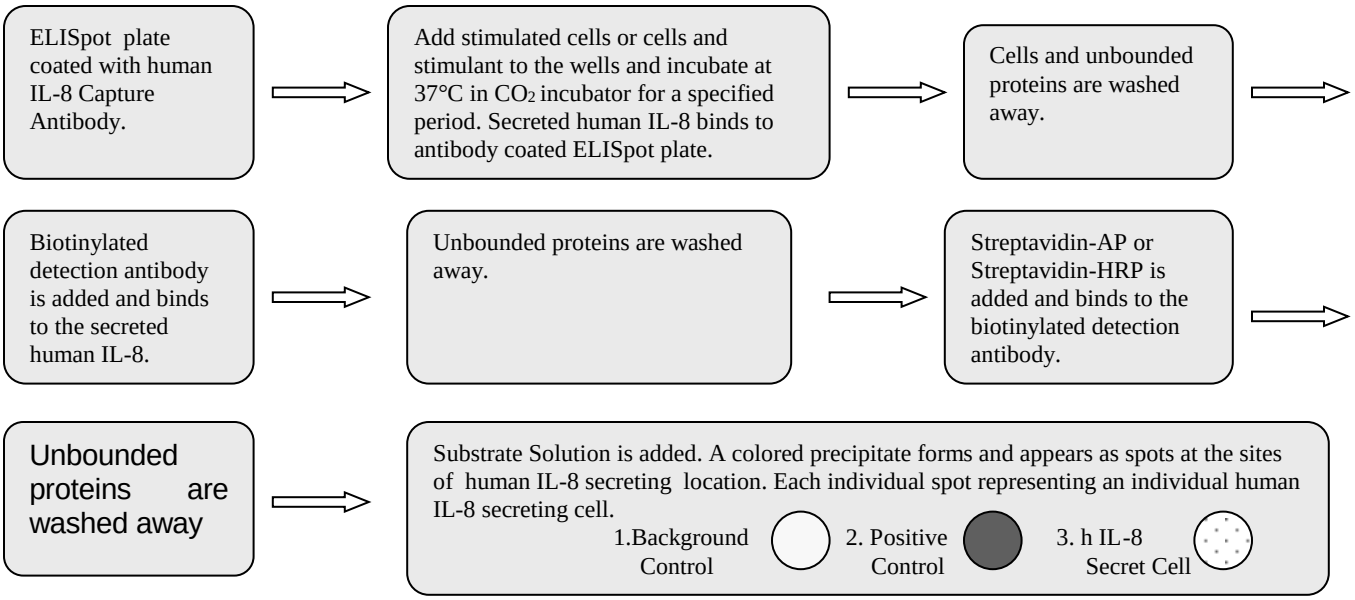
The IL-8 predominant form secreted by stimulated monocytes has 72 residues (MW=8385), whereas the predominant form secreted by IL-1 stimulated endothelial cells has 77 residues (MW=8922). These variants have similar biological activities, although the 72-residue form of IL-8 appears to be 2 to 10 fold more potent than the 77-residue form depending on the type of assay used (7).

Various non-infectious human diseases are known to be associated with neutrophilia and/or neutrophil infiltration into organs. Examples of some of these human diseases include rheumatoid arthritis, gouty arthritis, psoriasis, glomerulonephritis, adult respiratory distress syndrome, immune vasculitis, inflammatory bowel disease, ischemia-reperfusion syndrome (including myocardial infarction and

multiple organ failure), chorioretinitis, cystic fibrosis, septic shock, acute meningococcal infections, alcoholic hepatitis and mediterranean fever (8). The presence of IL-8 has been positively identified in gouty arthritis, psoriatic scale, plasma from adult respiratory syndrome caused by sepsis, and serum from nephrotic syndrome as well as in the joint fluids from rheumatoid arthritis. The peripheral blood mononuclear cells (PBMC) obtained from patients undergoing an asthmatic attack have been shown to spontaneously produce *in vitro* IL-8-like molecules. The production of IL-8 triggers many other activities that contribute to these human diseases; however, IL-8 is not known to trigger systemic inflammatory reactions such as fever, acute phase protein induction.

This ELISpot kit is developed to detect and visualize of single cells secreting human IL-8.

PRINCIPLES OF THE ASSAY



REAGENTS PROVIDED

All reagents provided are stored at -20°C. Refer to the expiration date on the label.

Name (Part No.)	Size	Description	Usage and Storage
1)Concentrated human IL-8 Capture Antibody (Part SL10008A-1)	1 Vial	Lyophilized mouse anti-human IL-8 monoclonal antibody	Stock Solution: Reconstitute Concentrated Human IL-8 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 IL-8 Detection Antibody (Part SL 10008A-2)	1 Vial	Concentrated Biotinylated mouse anti-human IL-8 monoclonal antibody	Stock Solution: Reconstitute Concentrated human IL-8 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 IL-8 (2ng/vial, Yes Biotech, Catalogue Number SL10008C) 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.
   Yes Biotech BCIP/NBT Substrate Solution for ELISpot (10mL/bottle, Yes Biotech, 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 IL-8 secretion from peripheral blood mononuclear cells (PBMCs) is as following:

1. Add $1x10^4$ – 5 x 10^4 /mL PBMCs in 50 ng / mL phorbol 12-myristate-13-acetate and 0.5 ug/mL calcium inomycine.
2. Incubate for 12-24 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 IL-8 Capture Antibody Working Solution
   As described in **REAGENT PROVIDED.**
2. Add 100 µL of Human IL-8 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 Yes Biotech IL-8 Positive Control (2ng/vial, Catalogue Number SL10008C) 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 IL-8 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 IL-8 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 IL-8 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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