

Human IL- 8 ELISpot Kit

For the quantitation of single cells releasing human IL-8.

Catalogue Number: SL10008E

96 tests

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



ANOGEN

2355 Derry Road East, Unit 23
Mississauga, Ontario
CANADA L5S 1V6

Tel: (905) 677-9221 or (877) 755-8324

Fax: (905) 677-0023

Email: info@anogen.ca ♦ Web Site: www.anogen.ca

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INTENDED USE

Human IL-8 enzyme-linked immunospot (ELISpot) whole kit with pre-coated PVDF - bottom Immunospot plates for the quantitation of single cells releasing human IL-8.

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

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 one 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 MGS/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-1 β 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, hepatocytes, chondrocytes, endothelial cells, glioblastoma cells, and mesothelial cells (6).

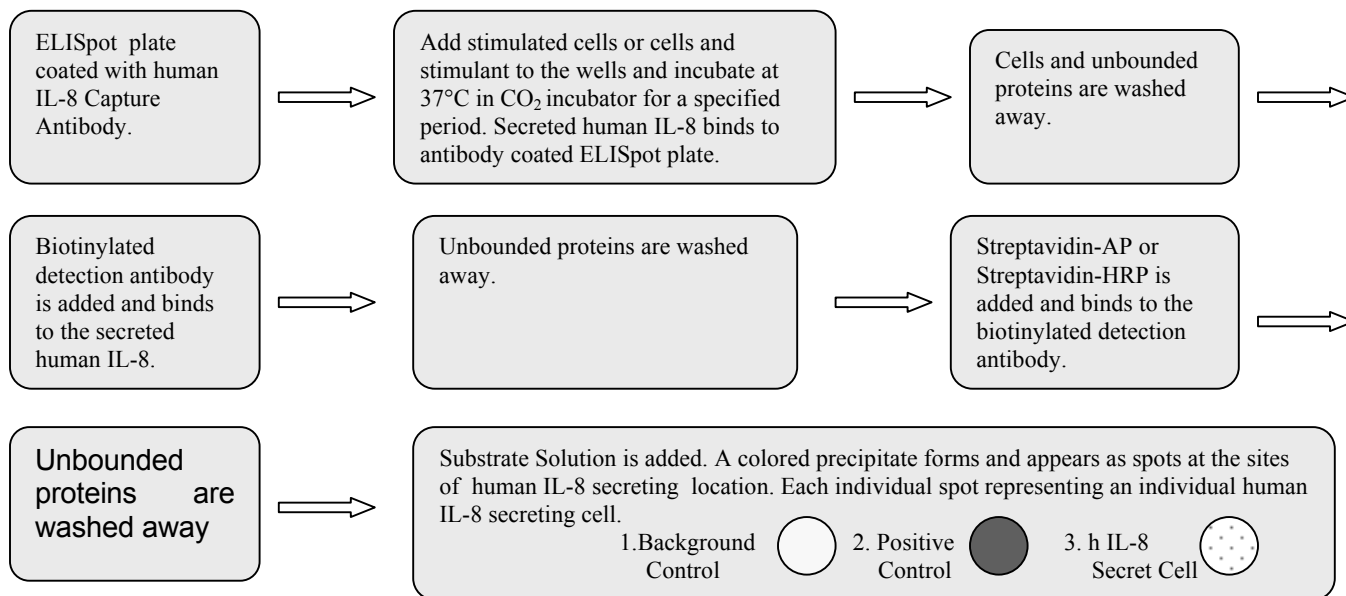
The IL-8 predominant form secreted by stimulated monocytes has 72 residues (MW=8385), whereas the predominant form secreted by IL-1 stimulated endothelial cells has 77 residues (MW=8922). These variants have similar biological activities, although the 72-residue form of IL-8 appears to be 2 to 10 fold 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 2.5 hours 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 4°C. Refer to the expiration date on the label.

Name (Part No.)	Size	Description	Usage and Storage
1) ELISpot Plates (1X 96tests, Part SL10008E-1)	1X 96tests	PVDF - bottom Immunospot plates pre-coated with mouse anti-human IL-8 monoclonal antibody.	Unpacked before use
2) Positive Control (Part SL10008E-2)	1 Vial	Lyophilized recombinant human IL-8 (2ng/vial)	Reconstitute 1 vial in 250 µL Cell Culture Media before use. Use in 1 hour. The final concentration is 8 ng/mL.
3) 20 X Wash Buffer Concentrated (Part	1 X 60mL	—	Add 1 volume of 20X Wash Buffer Concentrated to 19 volume of deionized water/distilled water. Use in

SL10008E-3)			1 week. Stored at room temperature.
4) Concentrated Human IL-8 Detection Antibody (Part SL 10008E-4)	1 Vial	120 μ L 100 x Concentrated Biotinylated mouse anti-human IL-8 monoclonal antibody	Add 1 volume of Human IL-8 Concentrated Detection Antibody to 100 volumes of Detection Antibody Diluent (Part SL 10008E-5) before use. Use in 1 month. Stored at 2-8 °C.
5) Detection Antibody Diluent (Part SL 10008E-5)	1 x 11mL	Protein with buffer and preservative.	Ready to use.
6) Concentrated Streptavidin - AP (Part SL 10008E-6)	1 Vial	120 μ L 100 x Concentrated Alkaline Phosphatase labeled Streptavidin.	Add 1 volume of Concentrated Streptavidin - AP to 100 volumes of Streptavidin – AP Diluent (Part SL 10008E-7) before use. Use in 1 month. Stored at 2-8 °C.
7) Streptavidin – AP Diluent (Part SL 10008E-7)	1 x 11mL	Protein with buffer and preservative.	Ready to use.
8) Substrate Solution (Part SL 10008E-8)	1 x 11mL	BCIP/NBT Substrate Solution.	Ready to use.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Pipettes with disposable tips, bottles, test tubes and racks, graduated cylinders, absorbent paper, and squirt bottle.
2. 37°C CO₂ incubator.
3. Deionized or distilled water.
4. Dissection microscope or ELISpot reader.

PRECAUTIONS

1. Allow kit reagents and materials to reach room temperature (20-25°C) 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 °C), 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 $1 \times 10^4 - 5 \times 10^4$ /mL PBMCs in 50 ng / mL phorbol 12-myristate-13-acetate and 0.5 μ g/mL calcium ionomycin.
2. Incubate for 12-24 hours at 37° C in CO₂ incubator.
3. Test according to this protocol.

ASSAY PROCEDURE

Aseptic Procedures: Steps 1 to 3 are aseptic procedures. Use sterile buffers and aseptic conditions, use laminar flow hood for procedures.

1. Wash 1 time with Cell Culture Media
Fill each well completely with sterile Cell Culture Media. Don't discard until cells are ready to be plated.
2. Prepare Positive Control
As described in **REAGENT PROVIDED**
3. Add 2 wells positive control, 2 wells negative control (unstimulated cells), 2 wells background control (sterile cell culture media) and IL-8 secreting cells with appropriate concentration to each plate, 100 μ L/well. Incubate at 37°C CO₂ 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.

4. Prepare 1x Wash Buffer, Human IL-8 Detection Antibody solution, and Streptavidin – AP solution.
As described in **REAGENT PROVIDED**.
5. Wash the plate 5 times with 1 x Wash Buffer
Decant or aspirate contents of the plate into a waste container. Fill each well completely with 1 x 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 hitting plate onto absorbent paper slightly.
6. Immediately add 100 μ L of Human IL-8 Detection Antibody to each well of the plate. Cover the plate and incubate 1hour at room temperature (20-25 °C).
7. Repeat wash procedure as described in step 5. Wash plate 5 times.
8. Immediately add 100 μ L of Streptavidin-AP to each well of the plate. Cover the plate and incubate 1hour at room temperature (20-25 °C).
9. Repeat wash procedure as described in step 5. Wash plate 5 times.
10. Immediately add 100 μ L 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.
11. Stop the assay

Rinse 5 times with deionized water/distilled water. After final wash, invert plate, and dry by hitting plate onto absorbent paper slightly.

13. Dry plate

Wet plates show higher background than completely dry plates. Remove the plastic underdrain from bottom 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.

14. Quantify spots using a dissection microscope or ELISpot reader.

15. Dried plate can be stored in sealed plastic bag in dark for 6 months.

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