

Human IL-6 ELISpot Kit

For the quantitation of single cells releasing human IL-6.

Catalogue Number: SL10023E

96 tests

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



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INTENDED USE

Human IL-6 enzyme-linked immunospot (ELISpot) whole kit with pre-coated PVDF - bottom Immunospot plates for the quantitation of single cells releasing human IL-6.

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INTRODUCTION

Interleukin 6 (IL-6) is a multifunctional protein that plays important roles in host defense, acute phase reactions, immune responses, and hematopoiesis. ⁽¹⁾ Synonyms for IL-6 include: B cell stimulatory factor-2 (BSF-2), ⁽²⁾ hybridoma/plasmacytoma growth factor, ⁽³⁾ hepatocyte stimulating factor, cytotoxic T cell differentiation factor and macrophage-granulocyte inducing factor 2A (MGI-2A).⁽¹⁾ IL-6 is expressed by a variety of normal and transformed cells including T cells, B cells, monocytes/macrophages, fibroblasts, hepatocytes, keratinocytes, astrocytes, vascular endothelial cells, mesangial cells, osteoblasts, carcinomas, sarcomas, myelomas, glioblastomas, and melanomas. The production of IL-6 is up-regulated by numerous signals including mitogenic or antigenic stimulation, lipopolysaccharide, calcium ionophore, IL-1, IL-2, IFN, TNF, PDGF, and viruses. IL-4 and IL-13 inhibit IL-6 expression in monocytes. ⁽⁴⁻⁶⁾

Natural human and murine IL-6 are glycoproteins containing N-and/or O-linked carbohydrates (human IL-6 contains two potential N-glycosylation sites, while mouse IL-6 has none). In comparison with mouse IL-6, human IL-6 exhibits approximately 65% sequence homology at the nucleotide level, and 42% homology at the amino acid level. Although human and mouse IL-6 are equally active on mouse cells, mouse IL-6 is not active on human cells.

IL-6 is predicted to have a four helix-bundle type tertiary structure found in a number of other cytokines including growth hormone, EPO, G-CSF, OSM, IL-11, CNTF, LIF, MGF, Prolactin, etc. ⁽⁷⁻⁹⁾ The gene structures of these cytokines also show varying degrees of relatedness. Based on these criteria, it has been suggested that these cytokines evolved from a common ancestral gene. Results of structure-function studies of IL-6 and other four α -helix bundle cytokines indicated that the c-terminal (helix D) regions of these cytokines are primarily responsible for binding to the receptors. ^(1,4)

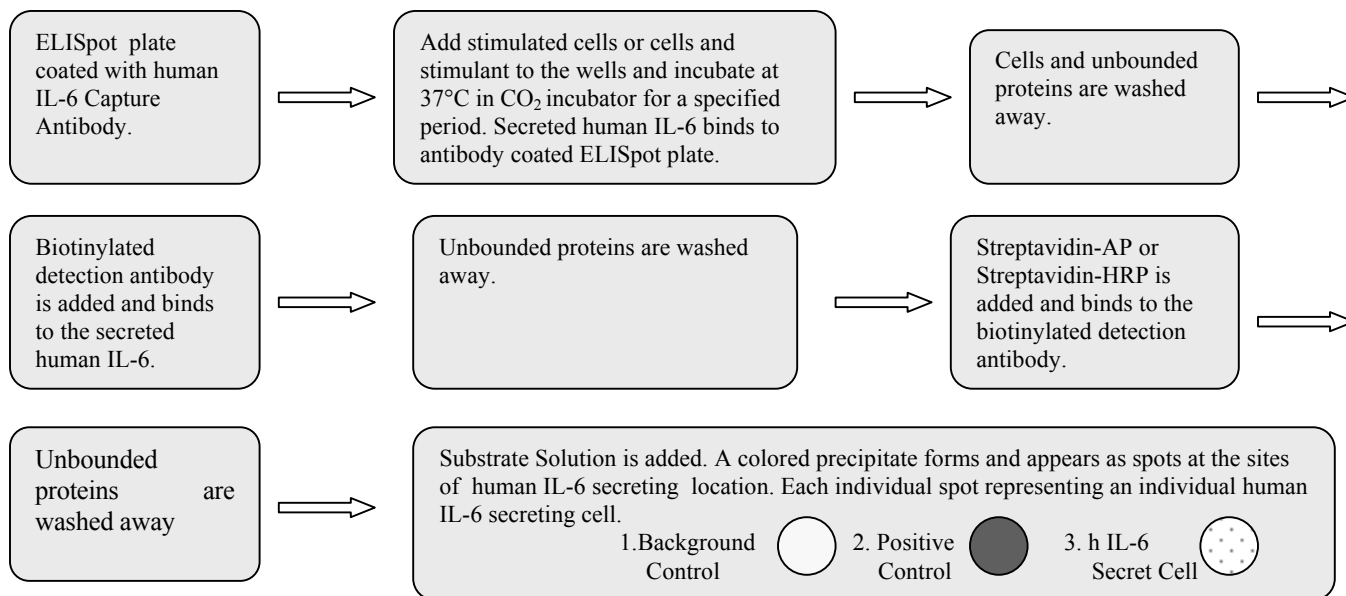
Interleukin 6 exerts multiple functions on numerous target cells. IL-6 plays an important role in immune functions. It has effects on B cell differentiation and antibody production, on cytotoxic T cell differentiation, on T cell activation, growth and differentiation, and on the induction of IL-2R α chain expression and IL-2 production in T cells. ⁽¹¹⁻¹³⁾ In hemopoiesis, IL-6 has blast cell growth factor activity and can synergize with IL-3 to shorten the G₀ period of early hemopoietic progenitors. ¹⁴⁾ In addition, IL-6 has been found to synergize with IL-3 in megakaryocyte development, increasing platelet numbers in vivo and the number, size and average ploidy value of megakaryocyte colonies formed from mouse or human bone marrow cells in vitro. ⁽¹⁵⁻¹⁸⁾ Similarly to IL-11 and LIF, IL-6 can induce the synthesis of hepatic acute phase proteins both in vivo and in vitro. ⁽¹⁹⁾ IL-6 has growth factor activities and will stimulate the growth of hybridomas, plasmacytomas, myelomas, sarcomas, ^(20, 21) carcinomas, ⁽²²⁾ EBV-transformed B cells, ⁽²³⁾ keratinocytes, and mesangial cells. In contrast to its

growth stimulatory activities, IL-6 is also a growth inhibitor for a number of leukemia and carcinoma cell lines. Additional bioactivities attributed to IL-6 include: induction of terminal differentiation of M1 myeloid leukemic cells; ⁽²⁴⁾ the differentiation and survival of neuronal cells; ^(25, 26) and the activation of osteoclast development. ⁽¹⁰⁾ Although IL-6 was also discovered as an antiviral factor produced by human diploid fibroblasts, the question of whether or not IL-6 has antiviral activity is controversial. Many groups have been consistently unable to find any antiviral activity for recombinant human IL-6. ⁽¹⁾

The various activities of IL-6 described above suggest that this factor will have a major role in the mediation of the inflammatory and immune responses initiated by infection or injury. Although the exact functions of IL-6 in vivo are not known, elevated IL-6 levels have been reported to be associated with a variety of diseases, including auto-immune diseases, mesangial proliferative glomerulonephritis, psoriasis, and malignancies such as plasmacytoma and myeloma. A great deal of work is currently in progress in order to provide a better understanding of the role of IL-6 in the modulation of normal and pathological processes.

This 2.5 hours ELISpot kit is developed to detect and visualize of single cells secreting human IL-6.

PRINCIPLES OF THE ASSAY



REAGENTS PROVIDED

All reagents provided are stored at 4°C. Refer to the expiration date on the label.

S7.5(00) IL-6 SL10023E

Name (Part No.)	Size	Description	Usage and Storage
1) ELISpot Plates (1X 96tests, Part SL10023E-1)	1X 96tests	PVDF - bottom Immunospot plates pre-coated with mouse anti-human IL-6 monoclonal antibody.	Unpacked before use
2) Positive Control (Part SL10023E-2)	1 Vial	Lyophilized recombinant human IL-6 (2ng/vial)	Reconstitute 1 vial in 250 μ L Cell Culture Media before use. Use in 1 hour. The final concentration is 8 ng/mL.
3) 20 X Wash Buffer Concentrated (Part SL10023E-3)	1 X 60mL	—	Add 1 volume of 20X Wash Buffer Concentrated to 19 volume of deionized water/distilled water. Use in 1 week. Stored at room temperature.
4) Concentrated Human IL-6 Detection Antibody (Part SL 10023E-4)	1 Vial	120 μ L 100 x Concentrated Biotinylated mouse anti-human IL-6 monoclonal antibody	Add 1 volume of Concentrated Human IL-6 Detection Antibody to 100 volumes of Detection Antibody Diluent (Part SL 10023E-5) before use. Use in 1 month. Stored at 2-8 $^{\circ}$ C.
5) Detection Antibody Diluent (Part SL 10023E-5)	1 x 11mL	Protein with buffer and preservative.	Ready to use.
6) Concentrated Streptavidin - AP (Part SL 10023E-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 10023E-7) before use. Use in 1 month. Stored at 2-8 $^{\circ}$ C.
7) Streptavidin – AP Diluent (Part SL 10023E-7)	1 x 11mL	Protein with buffer and preservative.	Ready to use.
8) Substrate Solution (Part SL 10023E-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 $^{\circ}$ 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-6 secretion from peripheral blood mononuclear cells (PBMCs) is as following:

1. Add 10⁴ /mL PBMCs in 3ug / mL phytohemagglutinin (PHA).
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-6 secreting cells with appropriate concentration to each plate, 100 µL/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 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-6 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.
12. 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.
13. Quantify spots using a dissection microscope or ELISpot reader.
14. Dried plate can be stored in sealed plastic bag in dark for 6 months.

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