

# Human IL-4 ELISpot Kit

For the quantitation of single cells releasing human IL-4.

Catalogue Number: SL10026E

*96 tests*

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

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## INTENDED USE

Human IL-4 enzyme-linked immunospot (ELISpot) whole kit with pre-coated PVDF - bottom Immunospot plates for the quantitation of single cells releasing human IL-4.

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## INTRODUCTION

Interleukin 4 (IL-4) was initially characterized as a B cell stimulatory factor (BSF-1) (1) and B-cell differentiation factor (BCDF $\gamma$ ) (2). It was subsequently revealed that IL-4 is a pleiotropic cytokine with multiple immune response modulating functions on diverse cell types including T cells, monocytes, macrophages, mast cells, fibroblasts, endothelial cells, osteoblasts, keratinocytes, hepatocytes and astrocytes (3-8). IL-4 is produced by CD4<sup>+</sup> TH0 and TH2 cells (9,10), fetal thymocytes (11), CD8<sup>+</sup> T cells (12), mast cells (13) and basophils (14).

Human and mouse cDNAs for IL-4 encode precursor proteins containing 153 and 140 amino acid residues, respectively. The signal peptides from the precursors are cleaved to yield mature proteins of 129 amino acid residues (human) and 120 amino acid residues (mouse) (17, 18). Both human and mouse proteins have multiple potential glycosylation sites and six cysteines that are all involved in intro-molecular disulfide bridges.

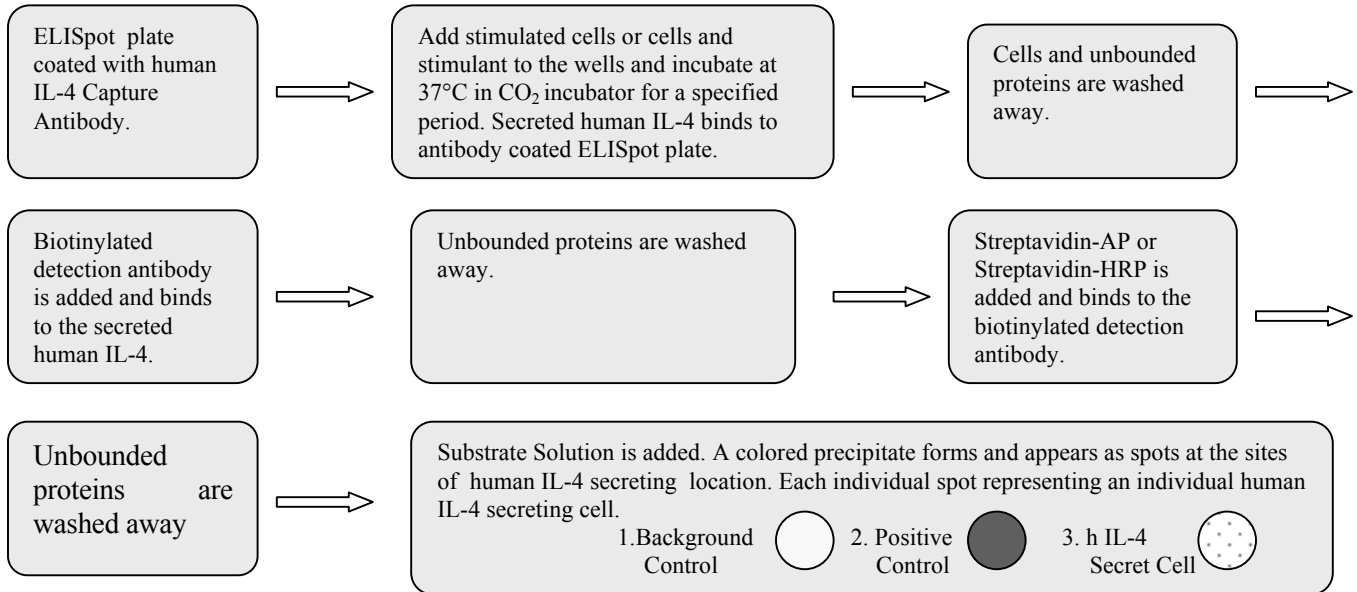
IL-4 exhibits approximately 25% amino acid sequence homology to IL-13 that shares a number of biological functions with IL-4 (19). The human and mouse IL-4 gene, each composed of four exons and three introns, have been localized to human chromosome 5q23-31 and mouse chromosome 11 in tandem with genes for IL-3, IL-5, IL-9, IL-13 and GM-CSF (20, 21).

IL-4 is an anti-inflammatory cytokine that exhibits multiple immuno-modulation functions on a variety of cell types, including T cells, B cells, monocytes, neutrophils, hematopoietic progenitors, fibroblasts, endothelial cells, and epithelial cells (15, 16).

The diverse effects exhibited by IL-4 *in vitro* suggest that it may play a central role in the modulation of immune and inflammatory responses *in vivo*. It was reported that introduction of malignant tumor cells transfected with the gene for IL-4 and producing IL-4 in athymic mice can block tumor formation by other transplantable tumor lines *in vivo*. Since the anti-tumor activity of IL-4 is evident in these athymic mice, IL-4-mediated host-defense responses other than T cell immunity must be involved (22). It was reported that IL-4 is important for protective immunity in parasitic nematode-infected mice, since IL-4 or IL-4R antibodies can blocked the polyclonal IgE response and abrogated protective immunity to the infection (23). In contrast to results obtained with the nematode-infected mice, endogenous IL-4 was reported to inhibit protective immunity in mice infected with the protozoan *Leishmania major* (24, 25). Clearly, much more work is required to unravel the complex network of IL-4-dependent processes in order to utilize IL-4 successfully in immunotherapy (16).

This 2.5 hours ELISpot kit is developed to detect and visualize of single cells secreting human IL-4.

## 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 SL10026E-1)	1X 96tests	PVDF - bottom Immunospot plates pre-coated with mouse anti-human IL-4 monoclonal antibody.	Unpacked before use
2) Positive Control (Part SL10026E-2)	1 Vial	Lyophilized recombinant human IL-4 (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 SL10026E-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) Human IL-4 Detection Antibody (Part SL 10026E-4)	1 x 11mL	Biotinylated mouse anti-human IL-4 monoclonal antibody	Ready to use.
5) Concentrated Streptavidin - AP (Part	1 Vial	120µL 100 x Concentrated	Add 1 volume of Concentrated Streptavidin - AP to 100 volumes of Streptavidin – AP Diluent (Part SL

SL 10026E-5)		Alkaline Phosphatase labeled Streptavidin.	10026E-6) before use. Use in 1 month. Stored at 2-8 °C.
6) Streptavidin – AP Diluent (Part SL 10026E-6)	1 x 11mL	Protein with buffer and preservative.	Ready to use.
7) Substrate Solution (Part SL 10026E-7)	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 CO<sub>2</sub> 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-4 secretion from peripheral blood mononuclear cells (PBMCs) is as following:

1. Add 5 x 10<sup>6</sup> /mL PBMCs in 3μg / mL phytohemagglutinin (PHA).
2. Incubate for 12-18 hours at 37° C in CO<sub>2</sub> 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-4 secreting cells with appropriate concentration to each plate, 100 µL/well. Incubate at 37°C CO<sub>2</sub> 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 µL of Human IL-4 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 µL 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 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.
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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