

Human IL-12 (p70)ELISpot Kit

For the quantitation of single cells releasing human IL-12.

Catalogue Number: SL10032E

96 tests

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

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INTENDED USE

Human IL-12 enzyme-linked immunospot (ELISpot) whole kit with pre-coated PVDF - bottom Immunospot plates for the quantitation of single cells releasing human IL-12.

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

INTRODUCTION

Interleukin 12 (IL-12), also known as natural killer cell stimulatory factor (NKSF) and cytotoxic lymphocyte maturation factor (CLMF), is a pleiotopic cytokine originally identified in the medium of cultured EBV-transformed RPMI-8866 cells) (1-3). IL-12 is a 75kDa glycoprotein heterodimer composed of two unequal, genetically-unrelated subunits. The smaller subunit (p35) has homology to IL-6 and G-CSF while the larger subunit (P40) shows recognizable similarity to the soluble receptor for IL-6, leading to the suggestion that IL-12 might have evolved from a cytokine/soluble receptor complex (2-6). Cells known to produce IL-12 include monocytes/macrophages, B cells and connective tissue type mast cells (7-10). IL-12 shows species specificity with human IL-12 reportedly showing minimal activity in the murine system (3,7). For reviews on IL-12, see references (8-12).

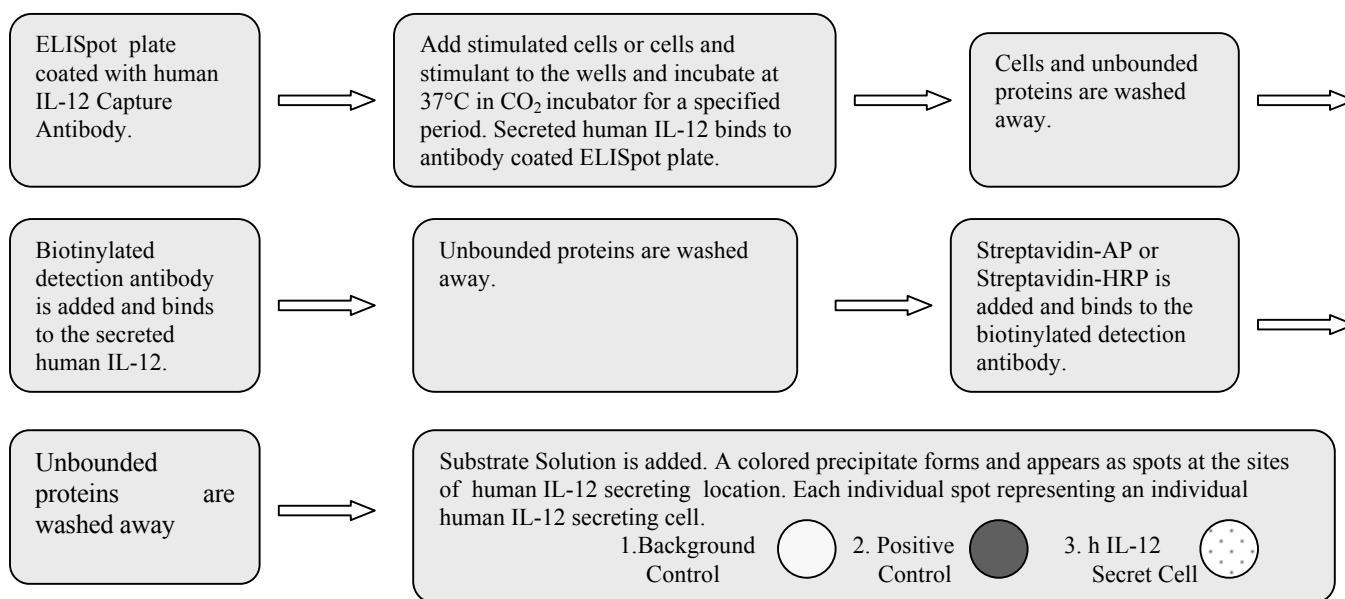
Each subunit of IL-12 apparently arises from a single copy gene. The transcription of the subunit of the subunit mRNAs is closely co-ordinated, although an excess of the larger subunit (P40) has been shown to be produced by B cells in addition to active IL-12 (1,3). Expression of the smaller chain (p35) is reported to be enhanced by simultaneous expression of the larger chain (p40). Although IL-12 activity cannot be demonstrated in the absence of either chain, the presence of only p40 is associated with inhibition of IL-12-associated activities (3,4). As suggested by their names, p35 has a native molecular weight of 35 kDa while p40 has a native molecular weight of 40 kDa. In humans, p35 is 197 amino acid residues in length with a predicted molecular weight of 22.5 kDa. The molecule contains 7 cysteine residues plus 3 potential N-glycosylation sites and the molecule is believed to be heavily glycosylated. The p40 subunit is 306 amino acid residues in length with a predicted molecular weight of 34.7 kDa. The molecule contains 10 cysteine residues and four potential N-linked glycosylation sites (3). The murine p35 subunit shows 60% sequence identity with the corresponding human subunit and is 193 amino acid residues in length with seven conserved cysteines and one possible N-linked glycosylation site. Murine p40 shows 70% sequence identity to human p40 and is 313 amino acid residues in length with eleven conserved cysteines and three potential N-linked glycosylation sites (7). In both human and mouse p35 and p40, the mature molecules separate functions can be attributed to p35 and p40. It is not clear what separate functions can be attributed to p35 and p40. Preliminary evidence suggests however that p40 is involved in receptor binding and p35 is important for signal transduction (13).

A unique high affinity receptor for IL-12 (IL-12R) has been characterized from PHA-stimulated human peripheral blood mononuclear cells (14). Approximately 110 kDa as determined by cross-linking studies, it has a K_d in the range of 100-600 pM (14). Cross-linking studies also suggested an association with a second protein of approximately 85 kDa. IL-12 receptor has also been reported to be present on PHA or IL-2 stimulated CD4+, CD8+, and CD56+ cells and on one T cell and one NK cell line (14, 15).

IL-12 is produced by macrophages and B lymphocytes and has been shown to have multiple effects on T cells and natural killer (NK) cells (16, 17). These include inducing production of IFN- γ and TNF by resting and activated T and NK cells, synergizing with other IFN- γ inducers at both the transcriptional and post-transcriptional levels to induce IFN- γ gene expression, enhancing the cytotoxic activity of resting NK and T cells, inducing and synergizing with IL-2 in the generation of lymphokine-activated killer (LAK) cells, acting as a comitogen to stimulate proliferation of resting T cells and inducing proliferation of activated T and NK cells (16). Evidence indicates that IL-12 produced by macrophages in response to infectious agent, is a central mediator of the cell-mediated immune response by its actions on the development, proliferation, and activities of TH1 cells (8,9, 18,19). In its role as the initiator of cell-mediated immunity, it has been suggested that IL-12 has therapeutic potential as a stimulator of cell-mediated immune responses to microbial pathogens, metastatic cancer, and viral infections such as AIDS (8,9, 18-20).

This 2.5 hours ELISpot kit is developed to detect and visualize of single cells secreting human IL-12.

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 SL10032E-1)	1X 96tests	PVDF - bottom Immunospot plates pre-coated with mouse anti-human IL-12 monoclonal antibody.	Unpacked before use
2) Positive Control (Part SL10032E-2)	1 Vial	Lyophilized recombinant human IL-12 (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 SL10032E-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-12 Detection Antibody (Part SL 10032E-4)	1 x 11mL	Biotinylated mouse anti-human IL-12 monoclonal antibody	Ready to use.
5) Concentrated Streptavidin - AP (Part SL 10032E-5)	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 10032E-6) before use. Use in 1 month. Stored at 2-8 $^{\circ}$ C.
6) Streptavidin – AP Diluent (Part SL 10032E-6)	1 x 11mL	Protein with buffer and preservative.	Ready to use.
7) Substrate Solution (Part SL 10032E-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 $^{\circ}$ 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 $^{\circ}$ 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 $^{\circ}$ 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.

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-12 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 µL of Human IL-12 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.

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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