

Human IL-1 alpha ELISpot Kit

For the quantitation of single cells releasing human IL-1 alpha.

Catalogue Number: SL10040E

96 tests

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

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INTENDED USE

Human IL-1 α enzyme-linked immunospot (ELISpot) whole kit with pre-coated PVDF - bottom Immunospot plates for the quantitation of single cells releasing human IL-1 α .

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

INTRODUCTION

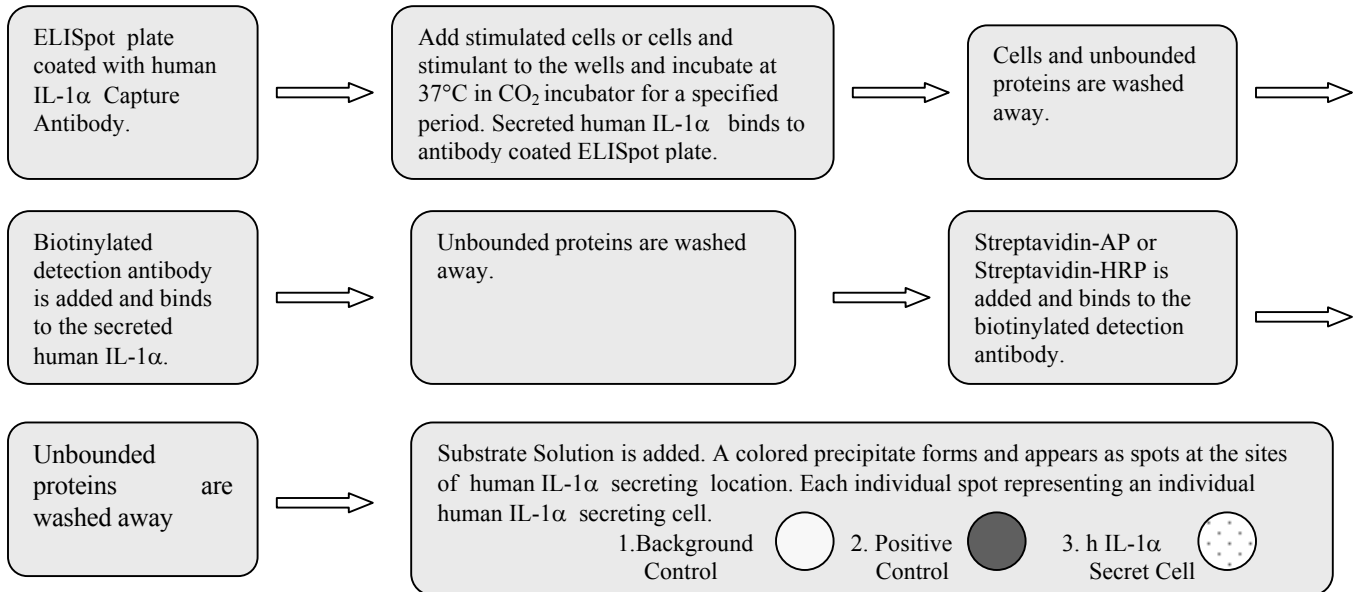
IL-1 α is a member of interleukin 1 family. IL-1 α and IL-1 β recognize the same IL-1 receptor and share a number of similar biological functions. IL-1 α is predominantly a cell-associated molecule whereas IL-1 β is a secreted molecule. IL-1 α is synthesized primarily as a 31 kDa precursor that lacks a signal peptide. Cleavage of the precursor is via the cysteine protease calpain, resulting in a 17.5 kDa mature IL-1 molecule. Being active in the processed form, the IL-1 precursor is also biologically active via specific cell binding. A portion of the precursor is transported to the cell surface and associated with the cell membrane. Precursor IL-1 α can be released and cleaved by extracellular proteases when the cells die, and can also be cleaved by activation of the calcium-dependent, membrane-associated calpains. Nearly all microbes and microbial products induce the production of IL-1 α . Furthermore, IL-1 α can be produced in monocytes and other cells in the 31 kDa precursor state.

IL-1 α can act on macrophages or monocytes by inducing its own synthesis as well as the production of TNF and IL-6. IL-1 α induces the production of IL-2, IL-2 receptors, GM-CSF and IL-4 from activated T cells, stimulates B cell proliferation and maturation, and increases immunoglobulin synthesis. IL-1 α affects NK cell activation and LAK production associated with other cytokines, and induces prostaglandin synthesis in endothelial cells and smooth muscle cells, collagenase production in synovial cells, and cartilage and calcium resorption in bones.

Studies have shown a connection between IL-1 α and the pathogenesis of endometriotic lesions. The increased expression of both matrix-degrading MMP-1 and its major stimulatory cytokine IL-1 α in endometriotic lesions and the selective co-expression in the stroma of endometriotic foci clearly suggests the involvement of the IL-1 α molecule in the pathogenic mechanisms leading to local invasion and tissue destruction. Reports also indicate that the translation of the neurotransmitter gene only occurs after receiving IL-1 α stimulation. This effect was suppressed by co-stimulation with IL-1 receptor antagonist. High levels of IL-1 α are associated with sepsis, rheumatoid arthritis, inflammatory bowel disease, acute and chronic myelogenous leukemia, insulin-dependent diabetes mellitus, and atherosclerosis.

This 2.5 hours ELISpot kit is developed to detect and visualize of single cells secreting human IL-1 α .

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 SL10040E-1)	1X 96tests	PVDF - bottom Immunospot plates pre-coated with mouse anti-human IL-1 α monoclonal antibody.	Unpacked before use
2) Positive Control (Part SL10040E-2)	1 Vial	Lyophilized recombinant human IL-1 α (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 SL10040E-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-1 α Detection Antibody (Part SL 10040E-4)	1 x 11mL	Biotinylated mouse anti-human IL-1 α monoclonal antibody	Ready to use.
5) Concentrated Streptavidin - AP (Part SL 10040E-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 10040E-6) before use. Use in 1 month. Stored at 2-8 °C.

6) Streptavidin – AP Diluent (Part SL 10040E-6)	1 x 11mL	Protein with buffer and preservative.	Ready to use.
7) Substrate Solution (Part SL 10040E-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 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.

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-1 α 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 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-1 α 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.

REFERENCES

1. Abbas, A.K., et al.(1987) Am. J. Pathol. 129:26.
2. Aribia, M.B., et al.(1987) J. Immunol. 139:443.
3. Cho KJ., et al. (2005) Stem Cells. 23:383
4. Dayer, J.M., et al.(1986) J. Clin. Invest. 74:645.
5. Dejuna, E., et al.(1987) Blood 69:635.
6. Dinarello, C.A. and S.M. Wolff (1982) Am. J. Med. 72:799.
7. Dinarello, C.A. (1988) Blood Purif. 6:164.
8. Dinarello, C.A. (1988) FASEB J. 2:108.

9. Dinarello, C.A. (1991) *Blood* 77:1627.
10. Dinarello, C.A. and S.M. Wolff (1993) *New Engl. J. Med.* 328:106.
11. Eastgate, J.A., et al. (1988) *Lancet* 2:706.
12. G. Hudelist et al (2005) *Hum. Reprod.* Advance Access published online on March 3, 2005
13. Herrmann, F., et al. (1988) *J. Clin. Invest.* 81:1415.
14. Kaye, J., et al. (1984) *Lymphokine Res.* 3:175.
15. Kobayashi, Y., et al. (1990). *Proc. Natl Acad. Sci. USA* 87, 5548–5552
16. Kobayashi, Y. (1991) *Chem. Pharmaceut. Bull.* 39, 1513– 1517.
17. Kovacs, E.J., et al. (1989) *Cancer Res.* 49:940.
18. Levett, D., et al. (1986) *J. Immunol.* 136:340.
19. Lipsky, P.E., et al. (1983) *J. Immunol.* 130:2709.
20. Lomedico, P.T., et al. (1984). *Nature* 312, 458–462
21. March, C.J., et al. (1985). *Nature* 315, 641–645
22. Miller, A.C. (1994). *Tox. Lett.* 74, 177–184.
23. Mosley, B. (1987). *J. Biol. Chem.* 262, 2941–2944
24. Navarro, S., et al. (1989) *J. Immunol.* 142:4339.
25. Sapsolsky, R., et al. (1987) *Science* 238:522.
26. Watanabe, N. (1994). *Cytokine* 6, 597–601