

Human IL-2 ELISpot Kit

For the quantitation of single cells releasing human IL-2.

Catalogue Number: SL10025E

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

TABLE OF CONTENT

	Page
INTENDED USE	2
INTRODUCTION	2
PRINCIPLE OF THE ASSAY	2
REAGENTS PROVIDED	3
MATERIALS REQUIRED BUT NOT SUPPLIED	3
PRECAUTIONS	4
SAMPLE PREPARATION	4
ASSAY PROCEDURE	4
REFERENCES	5

INTENDED USE

Human IL-2 enzyme-linked immunospot (ELISpot) whole kit with pre-coated PVDF - bottom Immunospot plates for the quantitation of single cells releasing human IL-2.

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

INTRODUCTION

Interleukin 2 (IL-2) is a lymphokine synthesized and secreted primarily by T helper lymphocytes that have been activated by stimulation with certain mitogens or by interaction of the T cell receptor complex with antigen/MHC complexes on the surfaces of antigen-presenting cells. The response of T helper cells to activation is induction of the expression of IL-2 and receptors for IL-2 and, subsequently, clonal expansion of antigen-specific T cells. At this level IL-2 is an autocrine factor, driving the expansion of the antigen-specific cells. IL-2 also acts as a paracrine factor, influencing the activity of other cells, both within the immune system and outside of it. B cells and natural killer (NK) cells respond to IL-2 when properly activated. The so-called lymphocyte activated killer, or LAK cells, appears to be derived from NK cells under the influence of IL-2.

Human IL-2 is a glycoprotein with an apparent molecular weight of 15,000 - 18,000. Natural IL-2 is glycosylated and varying degrees of glycosylation apparently account for the observed range of molecular weights seen on SDS-PAGE. Human IL-2 is synthesized as a polypeptide of 153 amino acid residues. The first 20 amino acids represent a signal sequence that is cleaved to produce the mature factor. The mature protein contains three cysteine residues, two of which form a disulfide bond that is required for biological activity. Murine IL-2 is approximately 63% identical to human IL-2, but contains a unique stretch of repeated glutamine residues. There is marked species cross-reactivity as human IL-2 has been found to be active on murine cell lines. Cells known to produce IL-2 include thymocytes, gamma delta T cells, B cells, CD4+ and CD8+ T cells, and neurons plus astrocytes.

IL-2 is a factor produced and secreted primarily by activated T helper cells that acts as an autocrine factor driving the expansion of antigen-specific cells and as a paracrine factor influencing the activity of a number of other cells including B cells, NK cells and LAK cells. A simplified but useful view of these activities is of lymphocytes expanding under the influence of IL-2 and becoming the target of other cytokines that cause their functional differentiation.

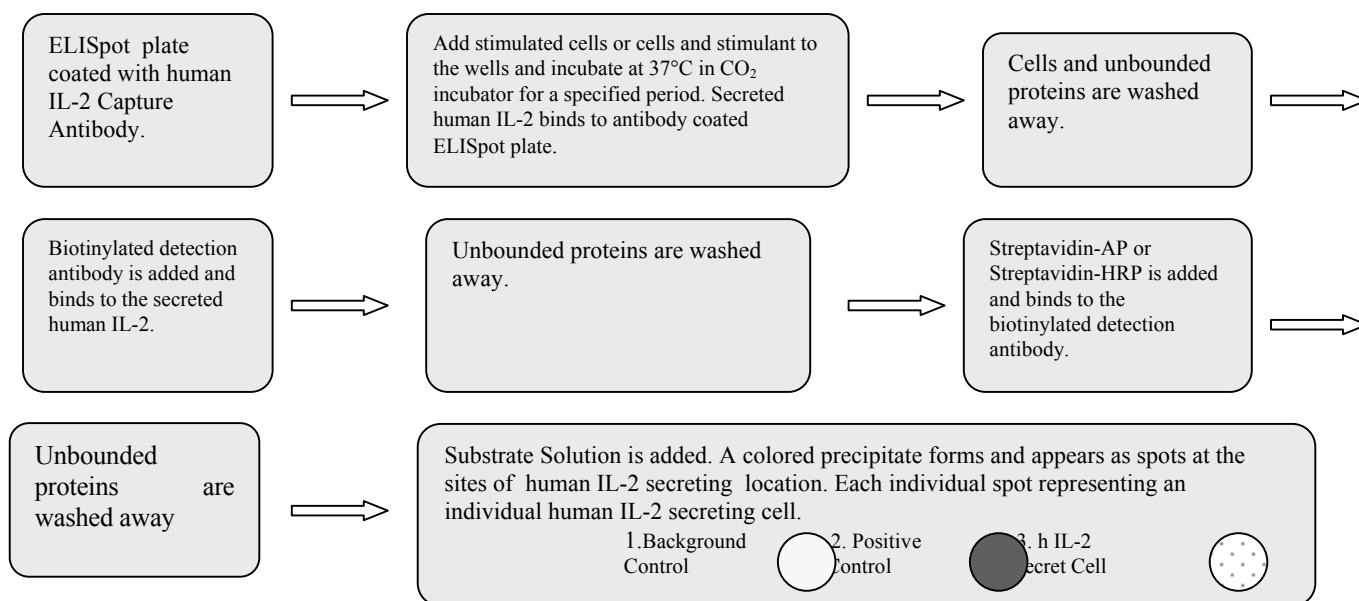
With respect to the specific role of IL-2 on the differentiation of T cells, the separation of CD4+ T helper cells into the categories of TH1 and TH2 according to their function in cell mediated or humoral immunity is a concept that is proving useful. In this system each category of cells secretes a characteristic set of cytokines that functions as a network to push the system either towards cellular immunity (delayed type hyper-sensitivity and cellular cytotoxicity) associated with TH1, or towards humoral immunity (antibody-mediated) associated with TH2. IL-2, along with IFN-gamma and TNF-beta, is a defining product of the TH1 subset. Although the TH1 and TH2 subsets are relatively clearly defined in the murine immune system, these categories are not so clear-cut in the human immune system where the designations TH1-like and TH2-like have been suggested.

Other cells under the possible influence of IL-2 are neutrophils, monocytes, and gamma delta T cells, all of which demonstrate either activation, augmented function, or increased survival when exposed to IL-2. Finally, it should be mentioned that IL-2 is finding its way, along with many other cytokines, into the neurosciences as a possible neuromodulator and growth regulator of glial cells.

Because of the central role of the IL-2/IL-2R system in mediation of the immune response, it is obvious that monitoring and manipulation of this system has important diagnostic and therapeutic implications. IL-2 has shown promise as an anti-cancer drug by virtue of its ability to stimulate the proliferation and activities of tumor-attacking LAK and TIL (tumor-infiltrating lymphocytes) cells. However, problems with IL-2 toxicity are still of concern and merit investigation.

This 2.5 hours ELISpot kit is developed to detect and visualize of single cells secreting human IL-2.

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 SL10025E-1)	1X 96tests	PVDF - bottom Immunospot plates pre-coated with mouse anti-human IL-2 monoclonal antibody.	Unpacked before use

2) Positive Control (Part SL10025E-2)	1 Vial	Lyophilized recombinant human IL-2 (4ng/vial)	Reconstitute 1 vial in 250 μ L Cell Culture Media before use. Use in 1 hour. The final concentration is 16 ng/mL.
3) 20 X Wash Buffer Concentrated (Part SL10025E-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-2 Detection Antibody (Part SL 10025E-4)	1 x 11mL	Biotinylated mouse anti-human IL-2 monoclonal antibody	Ready to use.
5) Concentrated Streptavidin - AP (Part SL 10025E-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 10025E-6) before use. Use in 1 month. Stored at 2-8 $^{\circ}$ C.
6) Streptavidin – AP Diluent (Part SL 10025E-6)	1 x 11mL	Protein with buffer and preservative.	Ready to use.
7) Substrate Solution (Part SL 10025E-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 $^{\circ}$ 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 quantify human IL-2 secretion from peripheral blood mononuclear cells (PBMCs) is as following:

1. Add 5×10^5 /mL PBMCs in 50 ng / mL phorbol 12-myristate-13-acetate and 0.5 ug/mL calcium ionomycine.
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-2 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-2 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.

REFERENCES

1. Academic Press Limited, London, pp.290-300.
2. Benveniste, E.N. and J.E. Merrill (1986) *Nature* 321:610.
3. Biron, C.A., et al. (1990) *J. Exp. Med.* 171:173.
4. Carswell, E.A., et al. (1975) *Proc. Natl. Acad. Sci.*, 72: 3666.
5. Conlon, K., et al.,(1995) *Eur. J. Immunol.* 25:644.
6. Dinarello, C. A. (1994) *Eur. Cytokine Netw.* 5:513.
7. Djeu, J.Y., et al. (1993) *J. Immunol.* 150:960.
8. El Bakkouri K, et al. (2005), *J Gene Med.* 7:189.
9. Espinoza-Delgado, I., et al. (1995) *J. Leukoc. Biol.* 57:13.
10. Grimm, E.A., et al. (1982) *J. Exp. Med.* 155:1823.
11. Hanisch, U-K., et al. (1993) *J. Neurosci.* 13:3368.
12. Hohmann, H.P., et al. (1989) *J Biol. Chem.* 264: 14927.
13. Hatakeyama, M. and T. Taniguchi (1990) "Interleukin-2" in *Peptide Growth Factors and Their Receptors I*, Sporn, M.B. and Roberts, A. B., eds. Springer-Verlag, New. York, p. 523
14. Henney, C.S., et al. (1981) *Nature* 291:335.
15. Jue, D.M., et al. (1990) *Biochem.* 29: 8371.
16. Kashima, K., et al. (1985) *Nature* 313:402.
17. Kelley, V.J., et al. (1988) *J. Immunol.* 140:59.
18. Kindler, V., et al. (1995) *Eur. J. Immunol.* 25:1239.
19. Kirkman, R.A., et al. (1985) *J. Exp. Med.* 162:358.
20. Kjeldsen-Kragh, J., et al. (1993) *Eur. J. Immunol.* 23:2092.
21. Kriegler, M., et al. (1988) *Cell.* 53:45.
22. Lotze, M.T., et al. (1981) *Cancer Res.* 41:4420.
23. Morgan, D.A., et al. (1976) *Science* 193:1007.
24. Mossman, T.R. and R.L. Coffman (1989) *Annu. Rev. Immunol.* 7:145.
25. Mozes, T., et al. (1991) *Immunol Lett.* 27: 157.
26. Nistico, G. and G. De Sarro (1991) *Trends Neurosci.* 14:146.
27. Olekowicz, L., et al. (1994) *Br. J. Haematol.* 88:892.
28. O'Malley BW Jr., et al (2005), *Laryngoscope.* 115:391
29. Ortaldo, J.R., et al. (1984) *J. Immunol.* 133:779.
30. Pfeffer, K., et al. (1993) *Cell.* 73: 457.
31. Pujol-Borrell, R., et al. (1987) *Nature* 326:304.
32. Robertson, M.J. and J. Ritz (1992). Chapter 12 in *NK Mediated Cytotoxicity.*
33. Rosenberg, S.A., et al. (1986) *Science* 233:1318.

34. Rosenberg, S.A., et al. (1987) *N. Eng. J. Med.* 316:889.
35. Rosenberg, S.A. (1988) *Ann. Surg.* 208:121.
36. Rubin, J.T. (1993) *Cancer Invest.* 11:460.
37. Shibuya, A., et al. (1993) *Blood* 81:1819.
38. Siegel, J.P., et al. (1987) *Science* 238:75.
39. Simpson, M.A. (1991) *Arch. Surg.* 126:717.
40. Smith, K.A. (1980) *Immunol. Rev.* 51:337.
41. Smith, K.A. (1984) *Annu. Rev. Immunol.* 2:319.
42. Smith, K.A. (1988) *Science* 240:1169.
43. Smith, K.A. (1993) *Blood* 81:1414.
44. Spagnoli, G.C., et al. (1993) *Cell. Immunol.* 146:391.
45. Swain, S.L. (1991) *Curr. Opin. Immunol.* 3:304.
46. Tadatsugu, T., et al. (1983) *Nature* 302: 305.
47. Tartaglia, L.A., et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:3535.
48. Tartaglia, L.A., et al. (1993) *Cell.* 73: 213.
49. Thomson, A. (1994) In *The Cytokine Handbook* (eds K.J. Tracey).
50. Tracey, K.J., et al. (1987) *Nature* 330:662.
51. Trinchieri, G., et al. (1984) *J. Exp. Med.* 160:1147.
52. Tsudo, M., et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:5394.
53. Tsukaguchi, K., et al. (1995) *J. Immunol.* 154:1786.
54. Waage, A., et al. (1987) *Lancet.* 1:355.
55. Waldmann, T.A., et al. (1984) *J. Exp. Med.* 160:1450.
56. Xin, Z., et al. (1994) *J. Neuroimmunol.* 54:59.
57. Yang, S.C., et al. (1991) *Cancer Res.* 51:3669.
58. Zubler, R.H., et al. (1984) *J. Exp. Med.* 160:1170.