

# Human IL-2 sR $\alpha$ ELISpot Kit

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

Catalogue Number: SL10033E

*96 tests*

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



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## INTENDED USE

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

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## INTRODUCTION

The biological function of IL-2 is obtained by binding to the specific interleukin-2 receptor (IL-2R). The IL-2R consists of three non-covalently linked chains, all of which are type I transmembrane proteins and include the  $\alpha$  chain (IL-2R $\alpha$ , p55),  $\beta$  chain (IL-2R $\beta$ , p75), and  $\gamma$  chain (IL-2R $\gamma$ , p65). The  $\alpha$  chain is cleaved from the cell surface via nonspecific proteolysis.

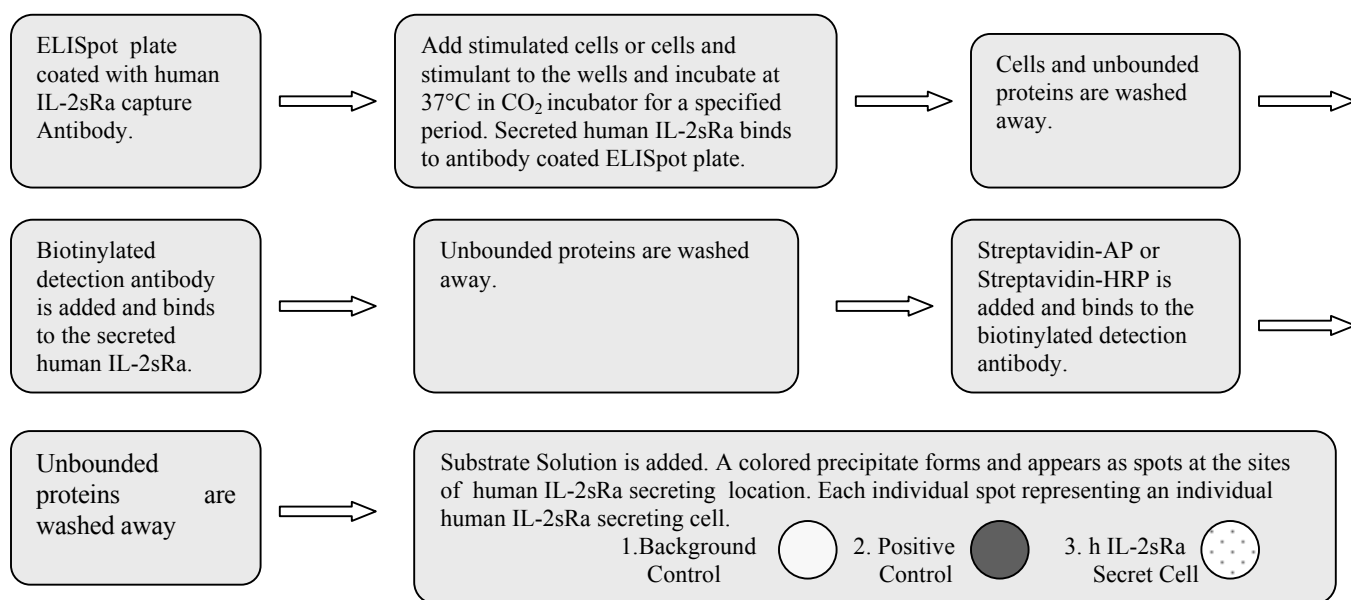
IL-2R $\alpha$  and IL-2R $\beta\gamma$  dimers bind to different residues on the IL-2 protein. The IL-2R $\alpha$  complex displays low affinity and the IL-2R $\alpha\beta$  complex displays intermediate affinity for IL-2 binding. Both IL-2R $\alpha$  and IL-2R $\alpha\beta$  complexes are unable to transduce a signal. The IL-2R $\beta\gamma$  complex has intermediate affinity for IL-2 binding and can transduce a signal with a relatively high concentration of IL-2. The IL-2R $\alpha\beta\gamma$  trimer is the high-affinity receptor for IL-2 and can transduce a signal successfully.

Many cells are capable of expressing IL-2R $\alpha$  including the antigen-activated T cells and B cells, and approximately 10% of natural killer (NK) cells, leukemia and lymphoma cells. When produced by activated T cells, the  $\alpha$  chain is 10-20 folds in excess of the  $\beta$  and  $\gamma$  chain. A soluble IL-2R $\alpha$  can be detected in tissue culture media of IL-2R $^+$  cells and in the serum of experimental animals and humans undergoing an immune response.

The major biological activities of IL-2R include promoting the proliferative expansion of T cells and NK cells upon activation, promoting the persistence of antigen-selected memory T cells, and promoting homeostasis of the immune system after it has successfully responded to an antigen. However, the biological activity of soluble IL-2R $\alpha$  is unclear. It has been reported that elevated IL-2 sR $\alpha$  level is accompanied by increased T and B cell activation and immune system activation as observed in rheumatoid arthritis, systemic lupus erythematosus (SLE), some leukemias and lymphomas. Because of its low affinity, IL-2 sR $\alpha$  would be expected to be an inhibitor of IL-2.

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

## 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 SL10033E-1)	1X 96tests	PVDF - bottom Immunospot plates pre-coated with mouse anti-human IL-2sRa monoclonal antibody.	Unpacked before use
2) Positive Control (Part SL10033E-2)	1 Vial	Lyophilized recombinant human IL-2sRa (3ng/vial)	Reconstitute 1 vial in 250 µL Cell Culture Media before use. Use in 1 hour. The final concentration is 12 ng/mL.
3) 20 X Wash Buffer Concentrated (Part SL10033E-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-2sRa Detection Antibody (Part SL 10033E-4)	1 x 11mL	Biotinylated mouse anti-human IL-2sRa monoclonal antibody	Ready to use.
5) Concentrated Streptavidin - AP (Part SL 10033E-5)	1 Vial	120µL 100 x Concentrated Alkaline Phosphatase labeled Streptavidin.	Add 1 volume of Concentrated Streptavidin - AP to 100 volumes of Streptavidin – AP Diluent (Part SL 10033E-6) before use. Use in 1 month. Stored at 2-8 °C.
6) Streptavidin – AP	1 x 11mL	Protein with buffer	Ready to use.

Diluent (Part SL 10033E-6)		and preservative.	
7) Substrate Solution (Part SL 10033E-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.

## 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-2sRa 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-2sRa 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. Chilosì, M., *et al.* (1987) Blood 70:1530.
2. Fernandez-Botran, R. (1991) FASEB J. 5: 2567.
3. Harrington, D.S., *et al.* (1988). Arch. Pathol. Lab Med. 112: 597.
4. Hatakeyama, M., *et al.* Science 244, 551-556.
5. Leonard, W.J., *et al.* (1994) Immunol Rev. 138: 61.
6. Leonard, W.J., *et al.* (1994) Curr. Opin. Immunol. 6: 631.
7. Minami, Y., *et al.* (1993). Annu. Rev. Immunol 11:245.
8. Pizzolo, G., *et al.* (1987) Br. J. Haematol. 67: 377.
9. Robb, R. J., *et al.* J. Exp. Med. 160, 1126-1146.

10. Rubin, L.A., *et al.* (1985) *Hybridoma* 4: 91.
11. Semenzato, G., *et al.* (1987) *Blood* 70: 396.
12. Sharon, M., *et al.* *Science* 234, 859-863.
13. Smith, K.A. (1989) *Annu. Rev. Cell Biol.* 5:397.
14. Steis, R.G., *et al.* (1988). *Blood* 71: 1304.
15. Takeshita, T., *et al.* *J. Immunol.* 148, 2154-2158.
16. Taniguchi, T and Y. Minami (1993) *Cell* 73: 5.
17. Teshigawara, K., *et al.* *J. Exp. Med.* 165, 223-238.
18. Tsudo, M., *et al.* *Proc. Natl Acad. Sci. USA* 83, 9694-9698.
19. Voss, S.D., *et al.* (1994) *Blood* 83: 626.
20. Wagner, D.K., *et al.* (1987) *J. Clin. Oncol.* 5:1262.
21. Waldmann, T.A. (1991) *J. Biol. Chem.* 266: 2681.
22. Waldmann, T.A. (1993) *Immunol. Today* 14: 264.
23. Wolf, R.E., *et al.* (1988) *Arthritis Rheum.* 31: 729.
24. Wang, H. M., *et al.* *J. Exp. Med.* 166, 1055-1069.