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INTENDED USE

Human IL-10 enzyme-linked immunospot (ELISpot) whole kit with pre-coated PVDF - bottom Immunospot plates for the quantitation of single cells releasing human IL-10.

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

INTRODUCTION

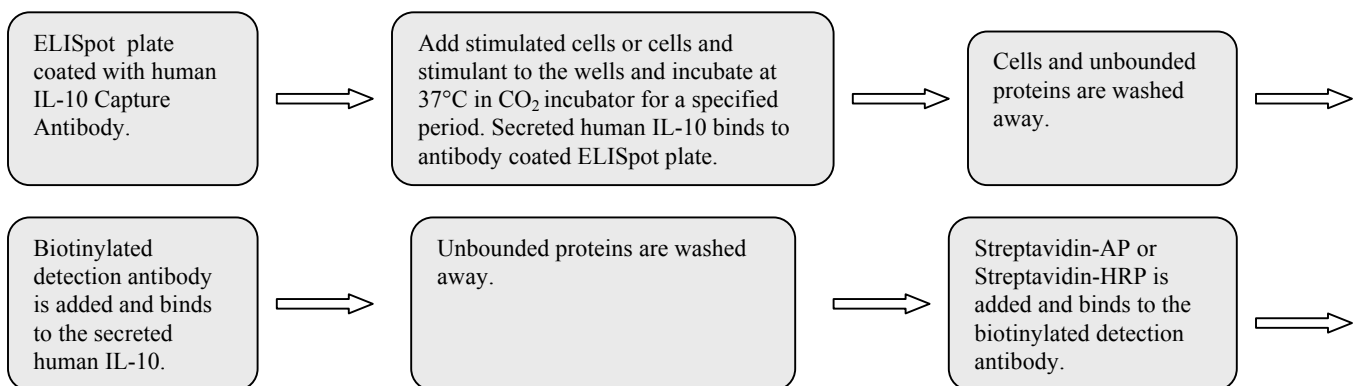
The mature human IL-10 is a protein with 160 amino acids and the functional IL-10 is a homodimer. IL-10 is expressed by a variety of cells, including mouse T cells (TH1, TH2, and Tr1 subsets), B cells derived from peripheral blood, tonsils or spleen, Epstein–Barr virus (EBV)-transformed B cell lines, Burkitt's lymphoma, AIDS lymphomas, monocytes, placental trophoblasts, bronchial epithelial cells, and certain tumor cells including melanomas and carcinomas of various origin.

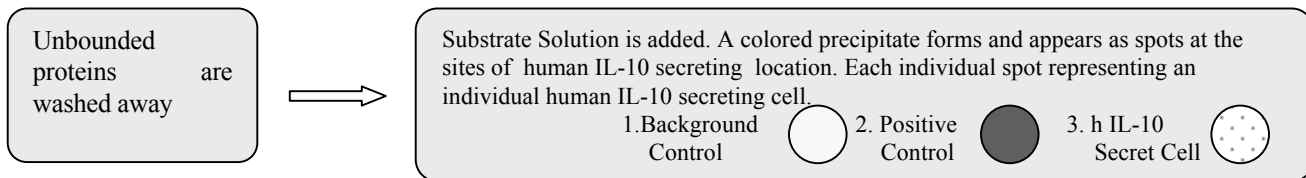
IL-10 plays an important role in inflammatory and immune responses. The biological activities of IL-10 include both immunosuppressive and immuno-stimulatory effects. For example, IL-10 can suppress the production of pro-inflammatory cytokines by monocytes and neutrophils, and down regulates the expression of activating and co-stimulatory molecules on monocytes and dendritic cells. IL-10 also can improve the growth of B cells and mast cells, and inhibit or enhance the activities of T cells depending on their activation conditions. The different polymorphisms in the IL-10 gene promoter have been demonstrated the association with both SIDS (sudden infant death syndrome) and sudden unexpected death due to infection.

Based on the functions of IL-10, it has been suggested that an IL-10 antagonist can be applied to boost anti-viral immunity against viruses such as EBV and that the IL-10 molecule itself may be used as an anti-inflammatory reagent.

This 2.5 hours ELISpot kit is developed to detect and visualize of single cells secreting human IL-10.

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 SL10027E-1)	1X 96tests	PVDF - bottom Immunospot plates pre-coated with mouse anti-human IL-10 monoclonal antibody.	Unpacked before use
2) Positive Control (Part SL10027E-2)	1 Vial	Lyophilized recombinant human IL-10 (2ng/vial)	Reconstitute 1 vial in 250 µL Cell Culture Media before use. Use in 1 hour. The final concentration is 16 ng/mL.
3) 20 X Wash Buffer Concentrated (Part SL10027E-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-10 Detection Antibody (Part SL 10027E-4)	1 x 11mL	Biotinylated mouse anti-human IL-10 monoclonal antibody	Ready to use.
5) Concentrated Streptavidin - AP (Part SL 10027E-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 10027E-6) before use. Use in 1 month. Stored at 2-8 °C.
6) Streptavidin – AP Diluent (Part SL 10027E-6)	1 x 11mL	Protein with buffer and preservative.	Ready to use.
7) Substrate Solution (Part SL 10027E-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.

A recommended method to quantify human IL-10 secretion from peripheral blood mononuclear cells (PBMCs) is as following:

1. Add 5×10^5 /mL unstimulated PBMCs.
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-10 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-10 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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