ab62937 – Human IL-10 ELISPOT Kit (with precoated plates)

Instructions for Use

For the qualitative measurement of IL-10 production and secretion in a single cell suspension.

This product is for research use only and is not intended for diagnostic use.

Version 4 Last Updated 29 January 2025

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1. BACKGROUND

Abcam's IL-10 Human ELISPOT kit is designed for the qualitative measurement of IL-10 production and secretion in a single cell suspension.

The ELISPOT assay involves a capture antibody highly specific for the analyte of interest coated to the wells of a PVDF bottomed 96 well microtitre plate, either during kit manufacture or in the laboratory. The plate is then blocked to minimize any non-antibody dependent unspecific binding and washed. Cell suspension and stimulant are added and the plate incubated allowing the specific antibodies to bind any analytes produced. Cells are then removed by washing prior to the addition of Biotinylated detection antibodies which bind to the previously captured analyte. Enzyme conjugated streptavidin is then added binding to the detection antibodies. Following incubation and washing substrate is then applied to the wells resulting in colored spots which can be quantified using appropriate analysis software or manually using a microscope.

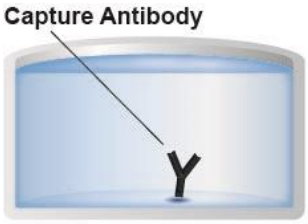
The ELISPOT assay is a highly specific immunoassay for the analysis of cytokine and other soluble molecule production and secretion from T-cells at a single cell level in conditions closely comparable to the *in-vivo* environment with minimal cell manipulation. This technique is designed to determine the frequency of cytokine producing cells under a given stimulation and the comparison of such frequency against a specific treatment or pathological state. The ELISPOT assay constitutes an ideal tool in the investigation of Th1 / Th2 responses, vaccine development,

viral infection monitoring and treatment, cancerology, infectious disease, autoimmune diseases and transplantation.

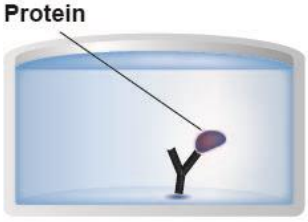
Utilising sandwich immuno-enzyme technology, Abcam ELISPOT assays can detect both secreted cytokines and single cells that simultaneously produce multiple cytokines. Cell secreted cytokines or soluble molecules are captured by coated antibodies avoiding diffusion in supernatant, protease degradation or binding on soluble membrane receptors. After cell removal, the captured cytokines are revealed by tracer antibodies and appropriate conjugates.

Interleukin-10 is a pleiotropic cytokine playing an important role as a regulator of lymphoid and myeloid cell function. Due to the ability of IL-10 to block cytokine synthesis and several accessory cell functions of macrophages this cytokine is a potent suppressor of the effector functions of macrophages, T-cells and NK cells. In addition, IL-10 participates in regulating proliferation and differentiation of B-cells, mast cells and thymocytes. The primary structure of human IL-10 has been determined by cloning the cDNA encoding the cytokine. The corresponding protein exists at 160 amino acids with a predicted molecular mass of 18.5 kDa. Based on its primary structure, IL-10 is a member of the four -helix bundle family of cytokines. In solution human IL-10 is a homodimer with an apparent molecular mass of 39 kDa. Although it contains an N-linked glycosylation site, it lacks detectable carbohydrates. Recombinant protein expressed in E. coli thus retains all known biological activities. The human IL-10 gene is located on chromosome 1 and is present as a single copy in the genome. The human IL-10 exhibits strong DNA and amino acid sequence homology to the murine IL-10 and an open reading frame in the Epstein- Barr virus genome, BCRF1 which shares many of the cellular cytokine's biological activities and may therefore play a role in the host- virus interaction. The immunosuppressive properties of IL-10 suggest a possible clinical use of IL-10 in suppressing rejections of grafts after organ transplantations. IL-10 can furthermore exert strong anti-inflammatory activities.

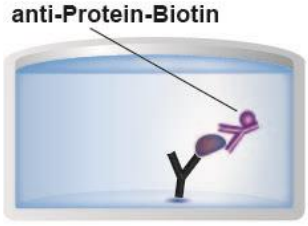
2. ASSAY SUMMARY



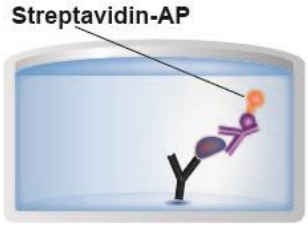
Equilibrate all reagents to room temperature. Prepare all the reagents and samples as instructed. 96-well PVDF bottomed plates are supplied pre-coated with capture antibody.



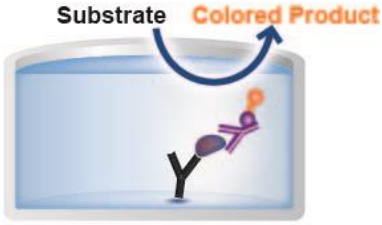
Add sample (Cells) to appropriate wells. Incubate at 37 ºC.



Aspirate and wash each well. Add prepared Biotinylated labeled detector antibody. Incubate at room temperature.



Add prepared Streptavidin-Alkaline Phosphatase mix to each well. Incubate at room temperature.



Add the substrate solution BCIP/NBT to each well and monitor spot formation.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at +2-8 °C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Quantity		Storage Condition (Before Preparation)
	1 x 96 tests	5 x 96 tests	
Pre-coated 96 well PVDF bottomed Microplate	1 x 96 wells	5 x 96 wells	+2-8ºC
Biotinylated Detection antibody	1 x 1 vial	1 x 1 vial	+2-8ºC
Streptavidin-Alkaline Phosphatase Conjugate	1 x 10 µL	1 x 50 µL	+2-8ºC
Bovine Serum Albumin (BSA)	1 x 2 g	1 x 2 g	+2-8ºC
Ready to use BCIP/NBT substrate buffer	1 x 11 mL	2 x 25 mL	+2-8ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Cell culture reagents (e.g. RPMI-1640, L-glutamine, FCS).
- Cell stimulation reagents (PMA, Ionomycin).
- 1X Phosphate Buffered Saline (PBS) (coating and wash step).

  For 1L of 10X PBS weigh out:

  80 g NaCl

  2 g KH_2PO_4

  14.4 g Na_2HPO_4 $2H_2O$.

  Add distilled water to 1L. Adjust the pH of the solution to 7.4 +/- 0.1. Dilute the solution to 1X before use.
- Miscellaneous laboratory plastic and/or glass, if possible sterile.
- CO_2 incubator.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
- Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

8. TECHNICAL HINTS

- Kit components should be stored as indicated. All the reagents should be equilibrated to room temperature before use.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross-contamination; for the dispensing of the Substrate solution, avoid pipettes with metal parts.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25ºC) prior to use.

9.1 **1% BSA PBS Solution (Dilution Buffer)**

For one plate dissolve 0.2 g of BSA in 20 mL of 1X PBS.

9.2 **Detection Antibody**

Reconstitute the lyophilised antibody with 550 µL of distilled water. Gently mix the solution and wait until all the lyophilised material is back into solution.

Dilute 100 µL of resuspended antibody into 10 mL Dilution Buffer and mix well.

If not used within a short period of time, reconstituted Detection Antibody should be aliquoted and stored at -20ºC. In these conditions the reagent is stable for at least one year. For optimal performance prepare the reconstituted antibody dilution immediately prior to use.

Please note for 1x96 demo kits, Biotinylated detection antibody is provided in liquid form.

9.3 **Streptavidin – AP conjugate**

For optimal performance prepare the Streptavidin-AP dilution immediately prior to use.

For 1 plate dilute 10 µL of Streptavidin-AP conjugate into 10 mL Dilution Buffer and mix well.

Do not keep this solution for further experiments.

10. CONTROL PREPARATION

Cells can either be stimulated directly in the antibody coated wells (Direct) or, first stimulated in 24 well plates or a flask, harvested, and then plated into the coated wells (Indirect).

The method used is dependent on 1) the type of cell assayed 2) the expected cell frequency. When a low number of cytokine producing cells are expected it is also advised to test them with the direct method, however, when this number is particularly high it is better to use the indirect ELISPOT method.

All the method steps following stimulation of the cells are the same regardless of the method (direct/indirect) chosen.

10.1 Positive Assay Control, IL-10 production

We recommend using the following polyclonal activation as a positive control in your assay.

Dilute CD4+ cells in culture media (e.g. RPMI 1640 supplemented with 2 mM L-glutamine and 10% heat inactivated fetal calf serum) containing 1 ng/mL PMA and 500 ng/mL Ionomycin.

Distribute 2.5 x 10^4 to 5 x 10^4 cells per 100 µL in required wells of an antibody coated 96-well PVDF plates and incubate for 15-20 hours in an incubator.

For other stimulators incubation times may vary, depending on the frequency of cytokine producing cells, and should be optimised in each situation.

10.2 Negative Assay Control

Dilute CD4+ cells in culture media to give an appropriate cell number (same number of unstimulated cells as stimulated sample cells) per 100 µL with no stimulation.

11. SAMPLE PREPARATION

- Dilute CD4+ cells in culture medium and stimulator of interest (i.e. sample, vaccine, peptide pool or infected cells) to give an appropriate cell number per 100 µL.
- Optimal assay performances are observed between 1 x 10^5 and 2.5 x 10^5 cells per 100 µL.
- Stimulators and incubation times can be varied depending on the frequency of cytokine producing cells and therefore should be optimised by the testing laboratory.

12. ASSAY PROCEDURE

12.1 Add 100 µL of PBS 1X to each well.

12.2 Incubate plate at room temperature for 10 minutes.

12.3 Empty the wells by flicking the plate over a sink and gently tapping on absorbent paper.

12.4 Add 100 µL of sample, positive or negative controls cell suspension to appropriate wells providing the required concentration of cells and stimulant (cells may have been previously stimulated).

12.5 Cover the plate and incubate at 37ºC in a CO_2 incubator for an appropriate length of time (10-15 hours).

Note: Do not agitate or move the plate during this incubation. The most appropriate incubation time for each experiment must be empirically determined by the end user as this can vary depending on the specific activation conditions, cell type and analyte of interest.

12.6 Empty the wells and remove excess solution then add 200 µL of PBS 1X to each well.

12.7 Incubate the plate at 4ºC for 10 minutes.

12.8 Empty the wells as previous and wash the plate 3x with 200 µL of PBS 1X.

12.9 Add 100 µL of diluted detection antibody to each well.

12.10 Cover the plate and incubate at room temperature for 1 hour 30 minutes.

12.11 Empty the wells as previous and wash the plate 3x with 200 µL of PBS 1X.

12.12 Add 100 µL of diluted Streptavidin-AP conjugate to each well.

12.13 Cover the plate and incubate at room temperature for 1 hour.

12.14 Empty the wells and wash the plate 3x with 200 µL of PBS 1X.

12.15 Peel off the plate bottom and wash both sides of the membrane 3 x under running distilled water. When washing is complete remove any excess solution by repeated tapping on absorbent paper.

12.16 Add 100 µL of ready-to-use BCIP/NBT buffer to each well

12.17 Incubate the plate for 5-15 minutes monitoring spot formation visually throughout the incubation period to assess sufficient color development.

12.18 Empty the wells and rinse both sides of the membrane 3x under running distilled water. Completely remove any excess solution by gentle repeated tapping on absorbent paper.

12.19 Allow the wells to dry and then read results. The frequency of the resulting colored spots corresponding to the cytokine producing cells can be determined using an appropriate ELISPOT reader and analysis software or manually using a microscope.

Note: spots may become sharper after overnight incubation at 4°C. Plate should be stored at room tempetature away from direct light, but please note color may fade over prolonged periods so read results within 24 hours.

13. TYPICAL SAMPLE VALUES

Reproducibility and Linearity

Intra-assay reproducibility and linearity were evaluated by measuring the spot development following the stimulation (PMA / Ionomycin) of 6 different CD4+ cells cell concentrations, 12 repetitions in 1 batch. The data shows the mean spot number, range and CV for the six cell concentrations.

Cells / Well	n	Mean number of spots per well	Minimum number of spots per well	Maximum number of spots per well	CV%
100,000	12	771	637	851	9
50,000 (recommended)	12	606	564	636	4
25,000 (recommended)	12	349	287	380	8
12,500	12	165	142	177	7
6,250	12	64	51	78	12
3,125	12	29	21	35	17

14. ASSAY SPECIFICITY

The assay recognizes native Human IL-10.

To define specificity of this ELISPOT, several proteins were tested for cross reactivity. There was no cross reactivity observed for these proteins tested IL-1β, IL-12, IFNγ, IL-4, IL-6, TNFα, IL-8, IL-2 and IL-13. This testing was performed using the equivalent IL-10 antibody pair in an ELISA assay.

15. TROUBLESHOOTING

Please refer to **www.abcam.com/ELISAandReagents** for troubleshooting tips.

16. NOTES

For all technical and commercial enquires please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)

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