ab62936– Human IL-6 ELISpot Kit

For the quantitative determination of the frequency of human IL-6-producing cells.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab62936

Storage and Stability

The entire ELISpot kit may be stored at 2 to 8°C for up to 6 months from the date of shipment. Avoid repeated freeze-thaw cycles. For extended storage, it is recommended to store at 2 to 8°C.

Materials Supplied

Item	1 × 96 tests	5 × 96 tests	Storage Condition
Biotinylated Human IL-6 Detection antibody	100 µl	---	4°C
Biotinylated Human IL-6 Detection antibody (Lyophilised)	---	1 vial	4°C
Bovine Serum Albumin	2 g	2 g	4°C
Human IL-6 Pre-coated 96 PVDF-bottomed-well plates	1 units	5 units	4°C
Ready-to-use BCIP/NBT substrate buffer	11 mL	25 mL	4°C
Streptavidin - Alkaline Phosphatase conjugated	10 µl	50 µl	4°C

For 001PC Kits (1 x 96 tests): Volumes of reagents are sufficient for a total of 96 tests,
**Please note for 001 and 002 : detection antibody is provided in liquid form*

Materials Required, Not Supplied

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

- Miscellaneous laboratory plastic and/or glass, if possible sterile
- Cell culture reagents (e.g. RPMI-1640, L-glutamine, FCS)
- Cell stimulation reagents (e.g. PMA, Ionomycin)
- CO2 incubator
- Tween 20
- Phosphate Buffered Saline (PBS)

Sample and control preparation:

Cell Stimulation

Cells can either be stimulated directly in the antibody coated wells (Direct) or, first stimulated in 24 well plates or 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 cytokines 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 whatever the method (direct/indirect) chosen.

Positive Control Preparation

We recommend using the following polyclonal activation as a positive control in your assay. Dilute PBMC in culture medium (e.g. RPMI 1640 supplemented with 2mM L-glutamine and 10% heat inactivated foetal calf serum) containing 1 µg/ml LPS. Distribute $1x10^4$ to $2.5x10^4$ cells per 100 µl in required wells of an antibody coated 96-well PVDF plate 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.

Negative Assay Control

Dilute PBMC in culture medium to give an appropriate cell number (same number of unstimulated cells as stimulated sample cells) per 100 µl with no stimulation.

Sample

Dilute PBMC 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.

Reagent Preparation

1X Phosphate Buffered Saline (PBS). For 1 litre of 10X PBS, weigh-out:

- 80g NaCl,
- 2g KH_2PO_4
- 14.4g Na_2HPO4; $2H_2O$

Add distilled water to 1 litre. Dilute the solution to 1X before use - Check the pH of the 1X solution and adjust to required pH: 7.4 +/- 0.1.

0.05% Tween PBS Solution (Wash Buffer). For one plate, dilute 50 µl of Tween 20 in 100 ml of PBS 1X.

1% BSA PBS Solution (Dilution Buffer). For one plate, dissolve 0.2 g of BSA in 20 ml of PBS 1X.

Human IL-6 Detection Antibody. Reconstitute the lyophilised antibody with 0.55 ml of distilled water. Gently mix the solution and wait until all the lyophilised material is back into solution.

Δ Note: *For 001PC kits, detection antibody is provided in liquid form.*

If not used within a short period of time, reconstituted Detection Antibody should be aliquoted and stored at -20°C. In these conditions the reagent is stable for at least one year. For optimal performance prepare the reconstituted antibody dilution immediately prior to use. For one plate, dilute 100 µl of antibody into 10 ml of Dilution Buffer and mix well. To avoid nonspecific background, it is recommended to filter the working solution using a disposable syringe and a 0.2 µm filter disc.

Streptavidin – AP conjugate. For optimal performance, prepare the Streptavidin-AP dilution immediately prior to use. It is recommended to centrifuge the vial for a few seconds to collect all the volume at the bottom. For one plate, dilute 10 µl of Streptavidin-AP conjugate into 10 ml of Dilution Buffer and mix well. Do not keep this solution for further experiments. To avoid nonspecific background, it is recommended to filter the working solution using a disposable syringe and a 0.2 µm filter disc.

BCIP/NBT. The reagent is ready-to-use. It should be clear to pale yellow. If precipitates occur, filter the solution using a disposable syringe and a 0.2 µm filter disc.

Assay Procedure

1. Add 100 µl of PBS 1X to every well.
2. Incubate plate at room temperature for 10 minutes.
3. Empty the wells by flicking the plate over a sink and gently tapping on absorbent paper.
4. Add 100 µl of sample, positive, and negative controls cell suspension to appropriate wells providing the required concentration of cells and stimulant (cells may have been previously stimulated).
5. Cover the plate and incubate at 37°C in a CO_2 incubator for an appropriate length of time (15-20 hours).
   Δ Note: *Do not agitate or move the plate during this incubation.*
6. Empty the wells and remove excess solution then add 100 µl of Wash Buffer to every well. Incubate the plate at 4°C for 10 minutes.
7. Empty the wells as previous and wash the plate 3X with 100 µl of Wash Buffer.
8. Add 100 µl of diluted human IL-6 detection antibody to every well.
9. Cover the plate and incubate at room temperature for 1 hour 30 minutes.
10. Empty the wells as previous and wash the plate 3X with 100 µl of Wash Buffer.
11. Add 100 µl of diluted Streptavidin-AP conjugate to every well.
12. Cover the plate and incubate at room temperature for 1 hour.
13. Empty the wells and wash the plate 3X with 100 µl of Wash Buffer.
14. Peel off the plate bottom and wash both sides of the membrane 3X under running distilled water, once washing is complete, remove any excess solution by repeated tapping on absorbent paper.
15. Add 100 µl of ready-to-use BCIP/NBT buffer to every well.
16. Incubate the plate for 5-15 minutes monitoring spot formation visually throughout the incubation period to assess sufficient colour development.
17. 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.
18. Read Spots: 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.

Δ Note: *Spots may become sharper after overnight incubation at 4°C in the dark.*

Δ Note: *Plate should be stored at room temperature away from direct light, but please note that colour may fade over prolonged periods so read results within 24 hours.*

Performance Characteristics

Reproducibility and Linearity:

Intra-assay reproducibility and linearity were evaluated by measuring the spot development following the stimulation (LPS) of 5 different PBMC cell concentrations, 12 repetitions. The data show the mean spot number, range, and CV for the cell concentrations.

Cells / well	n	Mean number of spots per well	Min	Max	CV%
10000 recommended	12	467	439	533	5.9
5000	12	340	327	370	3.9
2500	12	207	190	225	4.7
12500	12	118	108	129	6.3
6250	12	64	54	76	10.4

Specificity

The assay recognizes natural human IL-6.

There was no cross reactivity observed for any protein tested (IL-1 alpha, IL-1 beta, IL-10, IL-12, IFN gamma, IL-4, TNF alpha, IL-8 and IL13).

Click here for more information on ELISpot: https://www.abcam.com/protocols/elispot-protocol

For technical support contact information, visit: www.abcam.com/contactus

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