ab281970 – Human IL-17A/F heterodimer cytokine ELISpot Antibody Pair

For the quantitative determination of the frequency of Human IL-17A/F-producing cells.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab281970

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	10×96 tests	Storage Condition
Human IL-17A/F Capture antibody	2 × 0.5 mL	-20°C
Biotinylated Human IL-17A/F detection antibody	2 vials	-20 °C

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
- Streptavidin-conjugated (e.g. Streptavidin-alkaline phosphatase)
- Bovine Serum Albumin (BSA)
- Substrate solution (e.g. BCIP/NBT)
- Ethanol
- Cell culture reagents (e.g. RPMI-1640, L-glutamine, FCS)
- Cell stimulation reagents (PMA, Ionomycin)
- CO2 incubator
- Tween 20
- Phosphate Buffered Saline (PBS)
- 96-well PVDF bottomed plates (we recommended Millipore plates catalogue # MSIPN4510, MSIPS4510 and M8IPS4510)

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 Assay Control, Human IL-17A/F production

We recommend using the following polyclonal activation as a positive control in your assay. Dilute CD4+ cells in culture medium (e.g. RPMI 1640 supplemented with 2mM L-glutamine and 10% heat inactivated foetal calf serum) containing 1 ng/ml PMA and 500 ng/ml ionomycin (Sigma, Saint Louis, MO). Distribute $1x10^5$ to $2.5x10^5$ 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 CD4+ 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 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

Reagent Preparation

1X Phosphate Buffered Saline (PBS) Coating buffer
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.
Skimmed milk in PBS 1X solution (Blocking Buffer). For one non-sterile plate, dissolve 0.2 g of dry skimmed milk in 10 ml of PBS 1X. For one sterile plate, dilute 5 ml of liquid milk in 5 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.
0.05% Tween PBS Solution (Wash Buffer). For one plate, dilute 50 µl of Tween 20 in 100 ml of PBS 1X.
35% Ethanol (PVDF Membrane Activation Buffer). For one plate, mix 3.5 ml of ethanol with 6.5 ml of distilled water.
Human IL-17A/F Capture Antibody. This reagent is supplied sterile, once opened keep the vial sterile or aliquot and store at -20°C. For optimal performance prepare the Capture Antibody dilution immediately before use. For one plate, dilute 100 µl of capture antibody in 10 ml of PBS 1X and mix well.
Human IL-17A/F 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. For one plate, dilute 100 µl of antibody into 10 ml of Dilution Buffer and mix well.
Streptavidin conjugate. Dilute in Dilution buffer according to the instructions of the supplier.
Substrate buffer. Ready to use.

Assay Procedure

1. For PVDF membrane activation, add 25 µl of 35% ethanol to every well
2. Incubate plate at room temperature for 30 seconds.
3. Empty the wells by flicking the plate over a sink and gently tapping on absorbent paper. Thoroughly wash the plate 3x with 100 µl of PBS 1X per well.
4. Add 100 µl of diluted capture antibody to every well.
5. Cover the plate and incubate at 4°C overnight.
6. Empty the wells as previous and wash the plate once with 100 µl of PBS 1X per well.
7. Add 100 µl of blocking buffer to every well.
8. Cover the plate and incubate at room temperature for 2 hours.
9. Empty the wells as previous and thoroughly wash 3x with 100 µl of PBS 1X per well.
10. 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).
11. Cover the plate and incubate at 37°C in a CO_2 incubator for an appropriate length of time (15-20 hours). Note: do not agitate or move the plate during this incubation
12. Empty the wells and remove excess solution then add 100 µl of Wash Buffer to every well.
13. Incubate the plate at 4°C for 10 minutes.
14. Empty the wells as previous and wash the plate 3x with 100 µl of Wash Buffer.
15. Add 100 µl of diluted Human IL-17A/F detection antibody to every well.
16. Cover the plate and incubate at room temperature for 1 hour and 30 minutes.
17. Empty the wells as previous and wash the plate 3x with 100 µl of Wash Buffer.
18. Add 100 µl of diluted Streptavidin-conjugate to every well.
19. Cover the plate and incubate at room temperature following the supplier's instructions.
20. Empty the wells and wash the plate 3x with 100 µl of Wash Buffer.
21. Peel of 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.
22. Add 100 µl of ready-to-use substrate buffer to every well.
23. Incubate the plate for 5-15 minutes monitoring spot formation visually throughout the incubation period to assess sufficient colour development.
24. 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.

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

Performance Characteristics

Reproducibility and Linearity:

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

Cells / well	n	Mean number of spots per well	Min	Max	CV%
200000 recommended	12	43	33	55	16
100000	12	47	40	56	11
50000	12	33	21	65	36
25000	12	17	12	26	28
12500	12	15	8	23	28
6250	12	11	4	18	41

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

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

Version 1 | 2021-Mar-23