Human IL-4 Precoated ELISpot Kit

Catalog Number: EXLH006

For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.

info@exreprotein.com

Product Description

The Human IL-4 Precoated ELISpot Kit is used for detecting the frequency of human IL-4-secreting cells. Human IL-4 is captured by the immobilized antibody coated on the PVDF plate and detected by the biotinylated IL-4 antibody followed by streptavidin-HRP and the TMB Membrane Substrate. Read this manual in its entirety before use.

Limitations

- For Research Use Only. Not Intended for Diagnostic or Therapeutic Use.
- Variations in temperature, incubation time or technique while performing the assay can affect results.
- Variations in collecting, processing and storing samples may affect sample values.
- Do not substitute kit components with reagents from other sources or lots.

Materials Provided, Storage and Reconstitution

Store the kit at 2-8˚C for up to 12 months. Do not use past kit expiration date.

Reagents	Quantity	Reconstitution
Biotinylated Antibody (100 µL)	1 vial	1:100 dilution with Dilution Buffer R (1X)
Streptavidin-HRP (100 µL)	1 vial	1:100 dilution with Dilution Buffer R (1X)
Cell Stimulant (50 tests)	1 bottle	Follow instructions on the vial.
Dilution Buffer R (1X) (10mL)	2 bottles	Ready-to-use
PBS Buffer (1L/pack)	1 pack	Dissolve in 1L deionized water
TMB Membrane Substrate (10 mL)	1 bottle	Ready-to-use
Pre-coated PVDF Plate	1 plate	Ready-to-use

Additional Required Supplies

- Cell culture medium (it is recommended to use serum-free medium or RPMI-1640 with 100 units/mL Penicillin, 100 µg/mL Streptomycin and 5-10% fetal calf serum)
- Adjustable pipettes and pipette tips
- Distilled or deionized water
- Lymphocyte separation medium
- CO_2 incubator
- Dissection microscope or automated ELISpot analyzer for counting spots

Precautions

- Store the kit according to the labels and instructions.
- Reagents should be at room temperature before use.
- Streptavidin-HRP and biotinylated antibody should be centrifuged briefly before use.
- Avoid cross-contamination: change pipette tips between between samples, and between reagents.
- Use separate reservoirs for individual reagents.
- Ensure reagents are mixed evenly by shaking or stirring. Avoid foaming of reagents.
- Add all reagents in the same order to the plate to ensure consistent reaction times for each step.
- Remove remaining wash buffer from wells completely after each wash step. Blot the inverted plate on clean filter paper or paper towels before adding the next reagent.
- Avoid damaging the PVDF well membrane:
    - Do not make direct contact with pipette tips.
    - Do not remove the plastic plate base during incubation or substrate development. The base may be gently removed after completion of the substrate incubation to facilitate adequate washing of the well membrane and minimize background.

Preparation of Reagents

1X PBS: Dissolve 1 pack of PBS instant buffer in 1L deionized water to prepare 1X solution.

Cell Stimulant: Add sterile PBS to reconstitute lyophilized powder according to the label on the vial.

Biotinylated Antibody: Dilute 1:100 with Dilution Buffer R (1X).

Streptavidin-HRP: Dilute 1:100 with Dilution Buffer R (1X).

Protocol

Day 1: Cell Activation (Aseptic Conditions)

1. Reagents and samples should be at room temperature before use. All samples and controls should be assayed at least in duplicate.
2. Add 200 µL of culture medium to wet the PVDF membrane in each well and incubate for 5-10 minutes at room temperature.
3. Aspirate the culture medium from the wells.
4. Add 100 µL of cell culture to the negative control wells
5. Add 100 µL of the cell suspension to each well. The cell concentration should be adjusted according to the experimental design with respect to the mitogen or antigen.
6. Add 10 µL of Cell Stimulant (antigen) to each well.
7. Cover the plate and incubate at 37°C, 5% CO_2 for 16-24 hours. The specific incubation time should be adjusted according to the cells and experimental design.

Day 2: Detection Steps (Aseptic Conditions Not Required)

1. Aspirate the wells. Add 200 µL of ice-cold deionized water to the well and place in a refrigerator at 2-8°C for 10 minutes to remove the cells by lysis.
2. Decant or aspirate the liquid from the wells. Add 260 µL of 1X Washing Buffer to each well with a 60 second soak. Remove the Washing Buffer by aspirating or decanting, invert the plate and blot it against clean paper towels. Wash the plate at total of 6 times. Complete removal of wash buffer at each step will improve performance.
3. Add 100 µL of diluted Biotinylated Antibody to each well. Cover the plate and incubate at 37°C for 1 hour.
4. Repeat washing step.
5. Add 100 µL of diluted Streptavidin-HRP to each well. Cover the plate and incubate at 37°C for 1 hour.
6. Repeat washing step.
7. Add 100 µL of TMB Membrane Substrate to each well. Protect from light and incubate at room temperature for 5-30 minutes. Monitor the spot development by checking the plate every 5-10 minutes.
8. Decant the liquid from the plate. Carefully remove the plastic plate base and wash the inside and outside of the experimental wells and the plastic plate base with deionized water 3-5 times. Shake off excess water, protect from light and allow to dry at room temperature before replacing the dry plate base.
9. Spots are counted manually using a dissection microscope or an automated ELISpot analyzer.