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User's Manual

IL17AF ELISA Kit

REF **DEIA3730**

Σ **96T**

RUO

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

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

IL17AF ELISA Kit is a sandwich enzyme immunoassay for the quantitative measurement of free IL17AF in the supernatant of activated cells.

General Description

Interleukin 17 (IL-17) is a pro-inflammatory cytokine produced by a subset of T helper cells that develops distinct from the Th1- and Th2- cell differentiation pathways. IL-17, also known as CTLA-8, stimulates induction of other pro-inflammatory cytokines TNF alpha, IL-1 beta, IL-6, and IL-8, and reports strongly suggest the involvement of IL-17 in several chronic inflammatory diseases such as rheumatoid arthritis, psoriasis and multiple sclerosis. TGFβ (differentiation) and IL-23 (expansion) are required for induction and maintenance of Th17 (IL-17 producing) cells, which in turn induce the other proinflammatory cytokines. IL-17 (~32 kDa) protein is produced and exists as a homo-dimer, has homology to a herpes virus early protein, is one of the six members (IL-17A-F) of this cytokine family, and is well characterized and highly expressed by activated effector memory T cells.

Principles of Testing

The IL17AF ELISA kit can be completed in one day using a sandwich ELISA protocol. The anti-IL-17F antibody is coated on a plate which then captures free IL-17AF. The amount of bound IL-17AF is detected by adding a second biotinylated anti-IL-17A antibody followed by HRP-conjugated streptavidin. ABTS substrate is then added and the concentration is determined by colorimetric detection in an ELISA plate reader.

Reagents And Materials Provided

1. 100× Capture Antibody (Contains 0.02% Sodium azide. Sodium azide is highly toxic.): 250 µL
2. 100× Detection Antibody (Contains 0.02% Sodium azide. Sodium azide is highly toxic.): 250 µL
3. BSA: 0.5 g × 4
4. 20× Wash Buffer: 50 mL
5. Coating Buffer: 25 mL
6. 10× Blocking Buffer: 20 mL
7. HRP-Conjugated Streptavidin: 10 µL
8. ABTS Substrate: 2 × 12 mL
9. Recombinant IL-17AF Standard: 1 vial
10. ELISA Strip 96 Well Plates: 96 well

Materials Required But Not Supplied

1. Distilled water

2. 96-well ELISA plate reader

Storage

Except Recombinant IL-17AF Standard stored at -20 °C, other reagents are stored at 4 °C.

Plate Preparation

	1	2	3	4	5	6	7	8	9	10	11	12
A	4000 ng/mL	4000 ng/mL	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
B	2000 ng/mL	2000 ng/mL	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
C	1000 ng/mL	1000 ng/mL	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
D	500 ng/mL	500 ng/mL	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
E	250 ng/mL	250 ng/mL	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
F	125 ng/mL	125 ng/mL	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
G	62.5 ng/mL	62.5 ng/mL	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
H	Blank	Blank	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample

Reagent Preparation

NOTE: The included buffers and reagents are optimized for use with this kit. Substitution with other reagents is not recommended and may not give optimal results.

1. 1× Wash Buffer: Prepare 1× Wash Buffer by diluting 20× Wash Buffer in distilled water. The diluted Wash Buffer may be stored at 4°C, however we recommend preparing fresh 1× Wash Buffer for each experiment.
2. 1× Blocking Buffer: Prepare 1× Blocking Buffer fresh prior to experiment. Dilute 10× Blocking Buffer in distilled water. Dissolve 0.5 g BSA in 50 mL of 1× Blocking Buffer in a sterile bottle.

Assay Procedure

This kit allows for the quantitative measurement of IL-17AF in a 96-well microtiter format. All 96-wells may be used at one time or you may only use the wells as required by your experimental design. Use of duplicate wells for each time point is recommended to obtain accurate results.

Appropriate Controls to Include

Following is a list of suggested controls to include with each analysis:

1. No capture antibody added to well
2. No supernatant added to well
3. No capture antibody or supernatant added to well

4. Positive control: use a cell line or tissue known to constitutively express IL-17AF or a recombinantly expressed IL-17AF
5. Negative control: use a cell line or tissue known to not express IL-17AF

ELISA Protocol

Note: This protocol is designed for a full 96-well assay. You can prepare lower volumes of reagents if you are not using the entire plate for one test.

1. Coating: Dilute 120 µL of Capture Antibody in 12 mL Coating Buffer. Pipet 100 µL of 1× Capture Antibody into each well (A1 through H1 and A2 through H2 for the standard and any of columns 3 through 12 for your samples), seal the plate and incubate overnight (12-24 h) at room temperature. Wash the coated wells twice with 300 µL of 1× Wash Buffer.
2. Blocking: Add 200 µL of prepared 1× Blocking Buffer (see Reagent Preparation) to each well to block the remaining reactive surface. Seal the plate and incubate for 30 min to 1 h at RT on a shaker set to 100 RPM.
3. Prepare IL-17A Standard: Quick spin down a Recombinant IL-17AF Standard vial and add 100 µL of sterile deionized H_2O. Gently vortex to dissolve. Allow the vial to sit for 15 min, then vortex again. Stock standard concentration is 1 µg/mL. The stock standard may be stored in 4°C for up to 60 days after reconstitution. Suggested dilution of stock standard: add 4 µL of stock standard (1 µg/mL) in to 996 µL of 1× blocking buffer, this makes 4000 pg/mL. Set up a standard curve following the directions below:

   Remove Blocking Buffer from wells by flicking into an appropriate waste container and gently tapping the plate face-down on paper towels. Replace with 100 µL of fresh prepared 1× Blocking Buffer in each well B1 through H1 and B2 through H2 for the standard.

   Pipette 200 µL of reconstituted Recombinant IL-17AF Standard (1000pg/mL) into wells A1 and A2. Transfer 100 µL from wells A1 and A2 in to wells B1 and B2.

   Mix wells B1 and B2 by pipetting.

   Transfer 100 µL from well B1 to C1 and B2 to C2.

   Continue this serial dilution process to wells G1 and G2. After mixing, discard 100 µL of solution from wells G1 and G2.

   Do not add standard to wells H1 and H2. These will serve as blanks.

NOTE: Sensitivity: 4000 pg/mL is a recommended starting dilution. By reducing the starting concentration, researchers can determine the lowest detection limit of IL-17AF protein in the test samples.

4. Samples: Pipet 100 µL of positive and negative controls and 100 µL test samples into the appropriate wells. Seal the plate and incubate plate 2h at RT on a shaker. Samples may be diluted or serially diluted using 1× Blocking Buffer. Users need to empirically determine the optimal concentrations of their test samples so that the readings fall within the curve of the protein standard.
5. Washing: Remove samples and control lysates and wash 4× with 300 µL of 1× Wash Buffer. Tap plate several times upside down to remove residual Wash Buffer after final wash.
6. Detecting Antibody: Dilute 120 µL of 100× Detection Antibody in 12 mL of 1× Blocking Buffer and add 100 µ L 1× Detection Antibody to each well. Seal the plate and incubate for 1h at RT on a shaker.
7. Washing: Remove antibody solution and wash wells 4× with 300 µL of 1× Wash Buffer. Tap plate upside down to remove residual Wash Buffer after final wash.
8. Secondary: Dilute 2.4 µL of HRP-Conjugated Streptavidin in 12 mL of 1× Blocking Buffer. Add 100 µL of

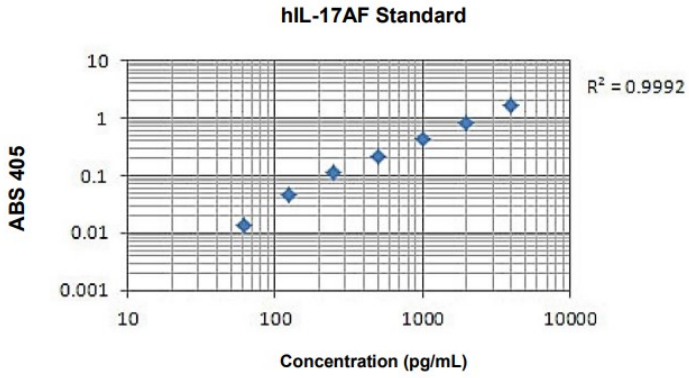
diluted secondary to each well. Seal the plate and incubate for 30 min at RT on a shaker.

9. Remove the secondary antibody and wash thoroughly (5×) with 300 µL of Wash Buffer letting the solution sit briefly between each wash. This ensures a thorough wash and lower background. Tap plate upside down several times to remove any residual Wash Buffer.
10. ABTS: Add 100 µL of ABTS Substrate to each well. Incubate the plate at RT for 15 to 30 min. Read the color development at 405 nm.

NOTE: Incubation time with ABTS Substrate may be increased or decreased depending on the concentration of samples. Most plate readers have a maximum reading of 2.0-3.0.

Typical Standard Curve

Following the provided protocol, human IL-17AF standard was titrated to determine detectible levels.



Sensitivity

4000 pg/mL is a recommended starting dilution. By reducing the starting concentration, researchers can determine the lowest detection limit of IL-17AF protein in the test samples.