ELISA PRODUCT INFORMATION & MANUAL

Human IL-17AF ActivELISA

NBP2-31047

Enzyme-linked Immunosorbent Assay for quantitative detection of Human IL-17AF. For research use only. Not for diagnostic or therapeutic procedures.

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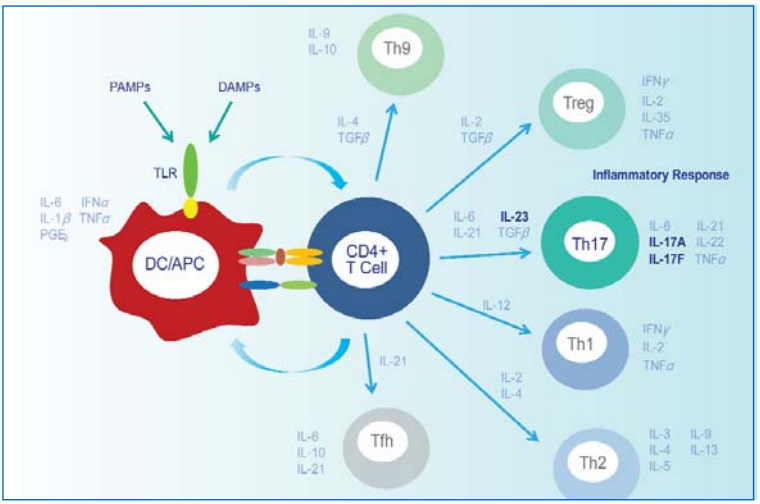
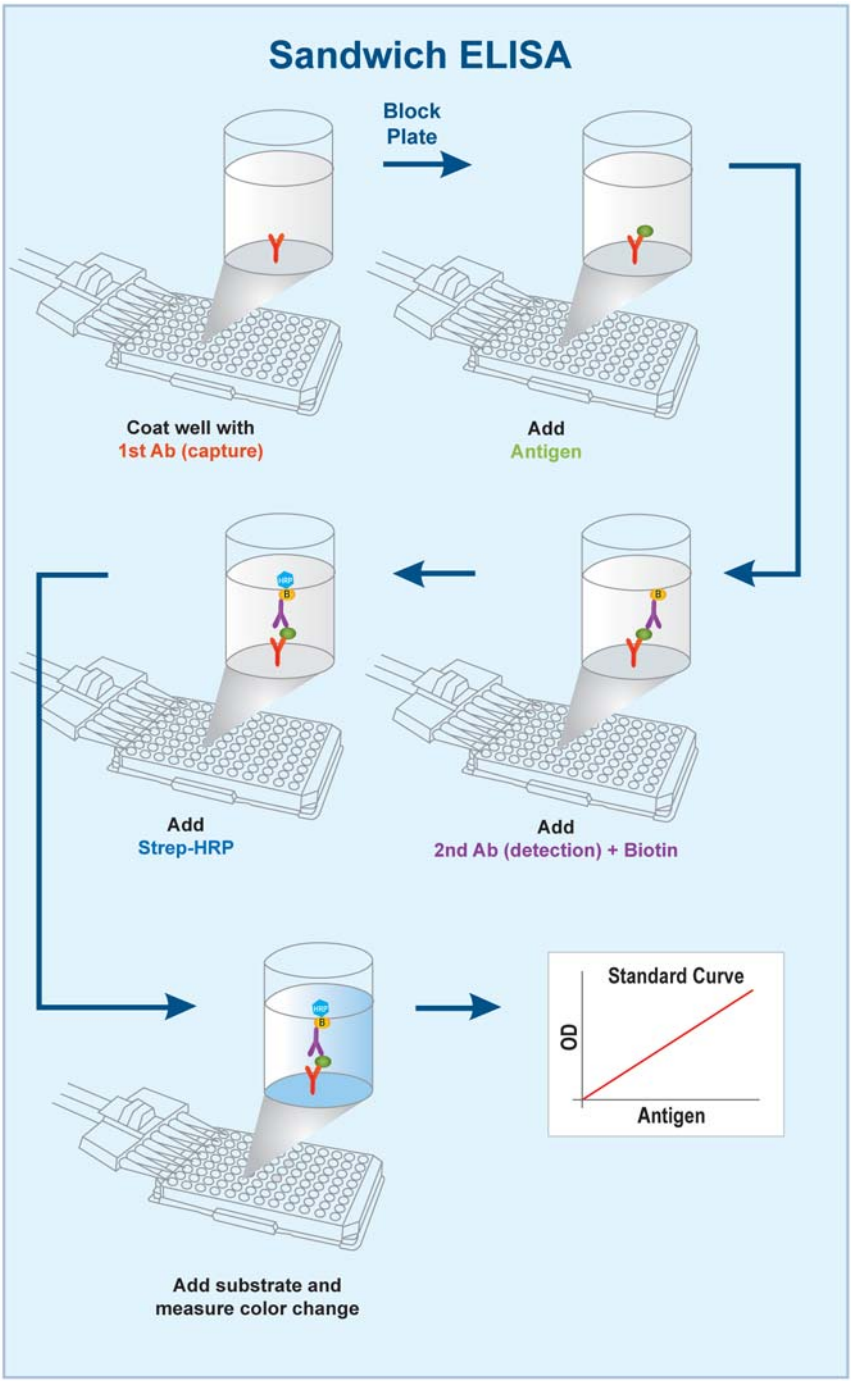
I. BACKGROUND

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. TGF-ß (differentiation) and IL-23 (expansion) are required for induction and maintenance of Th17 (IL-17 producing) cells, which in turn induce the other pro-inflammatory cytokines. IL-17 (~32kDa) 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.

II. OVERVIEW

The human IL-17AF ActivELISA™ Kit measures free IL-17AF in the supernatant of activated cells. Standard protocols for detecting IL-17AF activity include ELISA and Western Blot. The IL-17AF ActivELISA™ 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. *Specificity: Human*




Sandwich ELISA
Block Plate
Coat well with 1st Ab (capture)
Add Antigen
Add 2nd Ab (detection) + Biotin
Add Strep-HRP
Add substrate and measure color change
Standard Curve
OD
Antigen

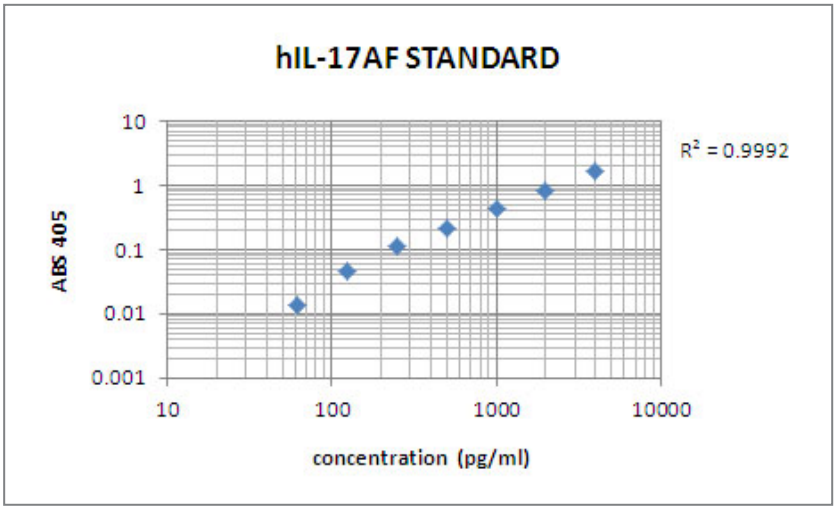
III. ADVANTAGES

- Multiple samples can be analyzed in a low-volume, high-throughput format.
- Full analysis complete in just hours

IV. EXPERIMENT

EXAMPLE STANDARD

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



V. KIT COMPONENTS AND STORAGE

Kit Components and storage for the NBP2-31047 IL-17AF ActivELISA™ Module.

REAGENTS (4°C STORAGE)		
KC-540-1	**100X Capture Antibody***	250 µl
KC-540-2	**100X Detection Antibody***	250 µl
KC-100	**BSA**	4 x 0.5 g
KC-101	**20X Wash Buffer**	50 ml
KC-141	**Coating Buffer**	25 ml
KC-142	**10X Blocking Buffer**	20 ml
KC-143	**HRP-Conjugated Streptavidin**	10 µl
KC-144	**ABTS Substrate**	2 x 12 ml
REAGENTS (-20°C, STORAGE--NON FROST-FREE FREEZER)		
KC-540-3	**Recombinant IL-17AF Standard**	1 vial
ADDITIONAL ITEMS INCLUDED		
ELISA Strip 96 Well Plates		2
NBP2-31047 Manual		1

** Contains 0.02 % Sodium azide. Sodium azide is highly toxic.*

Additional items required:

- Distilled water
- 96-well ELISA plate reader

VI. PREPARATION OF REAGENTS: IL-17AF ActivELISA™

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.*

1X Wash Buffer: Prepare 1X Wash Buffer by diluting 20X Wash Buffer (KC-101) in distilled water. The diluted Wash Buffer may be stored at 4°C, however we recommend preparing fresh 1X Wash Buffer for each experiment.

1X Blocking Buffer: Prepare 1X Blocking Buffer fresh prior to experiment. Dilute 10X Blocking Buffer (KC-142) in distilled water. Dissolve 0.5 g BSA (KC-100) in 50 ml of 1X Blocking Buffer in a sterile bottle.

VII. IL-17AF ActivELISA™ PROTOCOL

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 (KC-540-1) in 12 ml Coating Buffer (KC-141). Pipet 100 µl of 1X 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 1X Wash Buffer.

2. Blocking: Add 200 µl of prepared 1X Blocking Buffer (see Preparation of Reagents, page 7) 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-17AF Standard: Quick spin down the IL-17AF Standard vial (KC-541-3) and add 100 µl of sterile deionized H20. Gently vortex to dissolve. Allow the vial to sit for 15 min, then vortex again. Stock standard concentration is 1 ug/ml. The stock standard may be stored in 4°C for up to 60 days after reconstitution. Suggested dilution of stock standard: add 4 ul of stock standard (1 ug/ml) in to 996 ul of 1X 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 1X 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.

Table 1. Set up of a 96-well microtiter plate.

	Standard	Standard	Your Samples									
	1	2	3	4	5	6	7	8	9	10	11	12
A	4000 pg/ml	4000 pg/ml	-	-	-	-	-	-	-	-	-	-
B	2000 pg/ml	2000 pg/ml	-	-	-	-	-	-	-	-	-	-
C	1000 pg/ml	1000 pg/ml	-	-	-	-	-	-	-	-	-	-
D	500 pg/ml	500 pg/ml	-	-	-	-	-	-	-	-	-	-
E	250 pg/ml	250 pg/ml	-	-	-	-	-	-	-	-	-	-
F	125 pg/ml	125 pg/ml	-	-	-	-	-	-	-	-	-	-
G	62.5 pg/ml	62.5 pg/ml	-	-	-	-	-	-	-	-	-	-
H	Blank	Blank	-	-	-	-	-	-	-	-	-	-

**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 2 h at RT on a shaker. Samples may be diluted or serially diluted using 1X 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 4X with 300 µl of 1X Wash Buffer. Tap plate several times upside down to remove residual Wash Buffer after final wash.

6. Detection Antibody: Dilute 120 µl of 100X Detection Antibody (KC-540-2) in 12 ml of 1X Blocking Buffer and add 100 µl 1X Detection Antibody to each well. Seal the plate and incubate for 1 h at RT on a shaker.

7. Washing: Remove antibody solution and wash wells 4X with 300 µl of 1X Wash Buffer. Tap plate upside down to remove residual Wash Buffer after final wash.

8. Secondary: Dilute 2.4 µl of HRP-Conjugated Streptavidin (KC-143) in 12 ml of 1X 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 (5x) 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 (KC-144) 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.

VII. TROUBLESHOOTING IL-17AF ActivELISA™

Problem	Probable Cause	Suggestion
No signal	Failure to add all components.	Prepare a check-list and add the components in the correct order.
Low signal	Not enough supernatant per well.	Check the protein concentration. Add more Sup.
High background	Improper blocking.	Incubate with blocking buffer as recommended in the manual.
	Wells are not washed enough.	Wash plates thoroughly after each incubation.