# Human Interleukin 25, IL25 ELISA Kit

*Cat. No.: DEIA154*
*Lot. No.: (See product label)*
*Pkg.Size: 10 Plates*

## *INTENDED USE*

Human IL-17E ELISA development kit contains the key components required for the quantitative measurement of natural and/or recombinant IL-17E in a sandwich ELISA format within the range of 32-2,000 pg/ml. Using the ELISA protocol described below, this kit provides sufficient reagents to assay IL -17E in approximately 1,000 ELISA plate wells.

## *DESCRIPTION*

The protein encoded by this gene is a cytokine that shares sequence similarity with interleukin 17. This cytokine can induce NF-kappaB activation, and stimulate the production of interleukin 8. Both this cytokine and interleukin 17B are ligands for the cytokine receptor IL17BR. Studies of a similar gene in mice suggest that this cytokine may be a pro-inflammatory cytokine favoring the Th2-type immune response. Alternative splicing results in multiple transcript variants.

## *RECONSTITUTION & STORAGE*

***Capture Antibody:*** 100 µg of antigen-affinity purified goat anti-IL-17E. Centrifuge vial prior to opening. Reconstitute in 1.0 ml water for a concentration of 100 µg/ml. Following reconstitution the Capture antibodies may be stored at 2-8℃ for up to 6 months. For long term storage it is recommended to aliquot into working volumes and store at -70℃ in a manual defrost freezer.
***Detection Antibody:*** 25 µg of biotinylated antigen-affinity purified goat anti-IL-17E. Centrifuge vial prior to opening. Reconstitute in 0.25 ml sterile water for a concentration of 100 µg/ml. Following reconstitution the Detection antibodies may be stored at 2-8℃ for up to 6 months. For long term storage it is recommended to aliquot into working volumes and store at -70℃ in a manual defrost freezer.
***Human IL-17E Standard:*** 1 µg of recombinant IL-17E. Centrifuge vial prior to opening. Reconstitute in 1.0 ml sterile water for a concentration of 1 µg/ml. The Standard may be stored at 2 -8℃ for one month or aliquoted and stored at -70℃ for up to three months in a manual defrost freezer.
***UltraAvidin-HRP Conjugate:*** 40 µl vial. Upon receipt, UltraAvidin-HRP conjugate should be stored at 2-8℃, **DO NOT FREEZE.**
***TMB Liquid Substrate:*** Aspirate and wash plate 4 times. Add 100 µl of TMB HRPO Microwell Substrate Standard Kinetic One Component "Ready Use" to each well. Incubate at room temperature and monitorcolor development with an ELISA plate reader at 450 nm with wavelength correction set at 540 nm or 570 nm. The reaction may be stopped after 15-20 minutes by adding 100 µl of 2 M sulfuric acid to each well.

## *ANALYTE GENE INFORMATION*

***Gene Name:*** IL25 interleukin 25 [ *Homo sapiens* ]
***Official Symbol:*** IL25
***Synonyms:*** IL25; interleukin 25; interleukin 17E; IL17E; IL-25; IL-17E; Interleukin-25; UNQ3120/PRO10272; Interleukin-17E
***GeneID:*** 3565
***mRNA Refseq:*** NM_022789
***Protein Refseq:*** NP_073626
***MIM:*** 605658
***UniProt ID:*** Q9H293
***Chromosome Location:*** 14q11
***Function:*** cytokine activity

## *RECOMMENDED MATERIALS*

- ELISA microplates
- BSA
- Stop Solution 2 M Sulfuric Acid
- Dulbecco's PBS [10x]

## *RECOMMENDED SOLUTIONS*

***PBS:*** Dilute 10xPBS to 1xPBS, pH 7.2 in sterile water.
***Wash Buffer:*** 0.05% Tween-20 in PBS.
***Block Buffer:*** 1.0% BSA in PBS.
***Diluent:*** 1.0% BSA in PBS.

## *PLATE PREPARATION*

1. Dilute to 2.0 µg/ml of capture antibody and immediately add 100 µl to each ELISA plate well. Seal the plate and incubate overnight at room temperature.
2. Aspirate the wells to remove liquid and wash the plate 4 times using 300µl of wash buffer per well.
3. After the last wash invert plate to remove residual buffer and blot on paper towel.
4. Add 300µl block buffer to each well. Incubate for at least 1 hour at room temperature.
5. Aspirate and wash plate 4 times.



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## *ELISA PROTOCOL*

***Standard/Sample:*** Dilute standard from 2,000 pg/ml to zero in diluent. Immediately add 100 µl of standard or sample to each well in duplicate and incubate at room temperature for at least 2 hours.
***Detection:*** Aspirate and wash plate 4 times. Dilute a portion of detection antibody in diluent to a concentration of 50 ng/ml. Add 100 µl per well and incubate at room temperature for 2 hours.
***UltraAvidin-HRP Conjugate:*** Aspirate and wash plate 4 times. Dilute 3.0 µl of avidin-HRP conjugate 1:5,000 (this dilution factor may require some optimization) in diluent for a total volume of 15 ml. Add 100µl per well and incubate at room temperature for 30 minutes.
***TMB Liquid Substrate:*** Add 100 µl of TMB HRPO Microwell Substrate Standard Kinetic One Component "Ready Use" to each well. Incubate at room temperature for 20-30 minutes and monitor color development with an ELISA plate reader at 450 nm with wavelength correction set at 540 nm or 570 nm. Avoid placing plates in direct light.
***Stop Solution:*** The reaction may be stopped after 15-20 minutes by adding 100 µl of 2 M sulfuric acid to each well. All eye, hand, face, and clothing protection should be used when working with sulfuric acid.

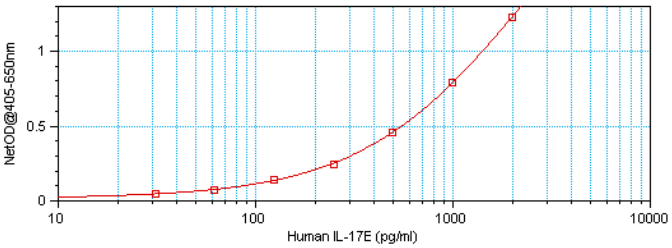
## *REFENRENCES*

1. Corrigan CJ, *et al*. Proc Natl Acad Sci U S A, 2011 Jan 25. PMID 21205894.
2. Hvid M, *et al*. J Invest Dermatol, 2011 Jan. PMID 20861853.

## *CROSS REACTIVITY*

When tested at 50 ng/ml the following antigen exhibited less than 5% cross reactivity: Human IL-17B, IFNγ, IL-8 (72aa), IL-8 (77aa), IL-10, IL-12, IL-12p40, IL-17A, D and F. Murine IL-17A and IL-17F.

## *TYPICAL STANDARD CURVE*




The standard curve is provided as an example only. A standard curve should be prepared with each assay.



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