Human Interleukin-17A ELISA kit
(DDXK-E-IL17A)

Intended use
The human IL17A ELISA kit is for quantitative determination of IL17A concentrations in human cell culture supernatants and biological fluids (plasma, serum). This kit is for research purpose only.

Background
IL17A (cytotoxic T lymphocyte associated antigen 8) is a $CD4^+$T cell-derived cytokine that stimulates stromal cells and macrophages to secrete proinflammatory cytokines. IL17A regulates the activities of NF-kappa B and mitogen-activated protein kinases. hIL17A stimulates epithelial, endothelial, and fibroblastic cells to secrete cytokines such as IL6, IL8, and G-CSF and PGE2. IL17A promoted the maturation of DC progenitors, as evidenced by increased cell surface expression of costimulatory molecules and MHC class II Ag, and allostimulatory capacity. IL17A had a lesser effect on the phenotype and function of more fully differentiated myeloid DC. These findings suggest a role for IL17A in allogeneic T cell proliferation that may be mediated in part *via* a maturation-inducing effect on DC. IL17A appears to be a novel target for therapeutic intervention in allograft rejection. High levels of this cytokine are associated with several chronic inflammatory diseases including rheumatoid arthritis, psoriasis and multiple sclerosis. (*Fossiez F. et al, 1996 ; J.Exp.Med*, *183:2593-2603* ; *Fossiez F. et al, 1998 ; Int. Rev. Immunol., 16:541-551).*

Kit contents
Capture Antibody: 0.5mg/mL of mouse anti-IL17A monoclonal antibody (clone 408H6.01). Dilute in 50mM carbonate buffer pH9.6 to a working concentration of 2.5µg/mL
Detection Antibody: 0.5mg/mL of HRPO-conjugated anti-IL17A mouse monoclonal antibody (clone 405B4.01). Dilute to a working concentration of 3µg/mL in PBS-1% BSA-0.05% Tween20
Standard: Each vial contains 1µg/mL of recombinant IL17A produced, purified and concentrated from eukaryotic cells. A 7-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 20ng/mL is recommended.

Storage
All the reagents should be aliquoted before storage. Minimize repeated freeze and thaw. Refer to expiration date on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot number.
-Standard storage: -80°C
-Capture and detection antibodies storage: -20°C

Materials and reagents required but not provided
96well-plate Nunc Maxi Sorp
50mM Carbonate buffer pH9.6
PBS-1% BSA-0.05% Tween20
TMB super sensitive HRP (TMBS100-0500, TEBU-BIO)
Multichannel pipettes and pipette tips
A standard microplate reader (620nm)

Sensitivity
The minimum detectable dose of human Interleukin-17 was determined to be approximately 1ng/ml.

Principle of the assay

The human Interleukin-17A ELISA kit is for the quantitative determination of human Interleukin-17A in human cell culture supernatants, plasma, serum, and various biological fluids.

This ELISA kit contains the specific components required for the development of human Interleukin-17A sandwich ELISAs. Each kit contains sufficient materials to run ELISAs on 3X96-well plates.

The DDX IL17A ELISA kit is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). The capture monoclonal antibody specific for IL17A is coated on a 96-well plate. Standards and samples are added to the wells, and any Interleukin-17A present binds to the immobilized antibody. The wells are washed and a HRPO-conjugated anti-Interleukin-17A monoclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". To produce color in proportion to the amount of Interleukin-17A present in the sample, TMB substrate solution is loaded and absorbance is measured at 620 nm.

Plate Preparation

Pre-warm all the reagents to room temperature prior to setting up the assay

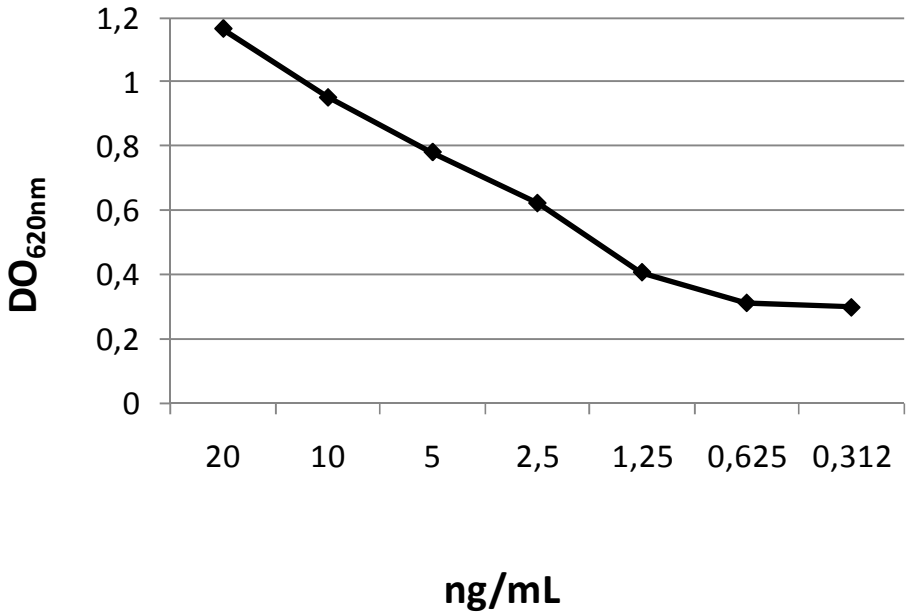
1. Dilute the capture antibody to 2.5µg/mL in 50mM carbonate buffer pH9.6. Immediately coat a 96-well microplate with 120µL per well of the diluted capture antibody. Seal the plate and incubate overnight at room temperature (RT).
2. Remove coating solution, and wash with at least 200µl of PBS-0.05% Tween20, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.

   The plates are now ready for sample addition.

Assay Procedure

1. Add 100 µL/well of sample or standards, diluted in PBS-1% BSA-0.05% Tween20. Seal the plate and incubate 1h30 at 37°C.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 µL/well of the detection antibody, diluted in PBS-1% BSA-0.05% Tween20 to 3µg/mL. Seal the plate and incubate 1h30 at 37°C.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 100 µL/well of TMB. Incubate for 5-10 minutes at RT.
6. Determine the optical density of each well, using a microplate reader set to 620 nm.

Example of standard curve



Troubleshootings

To obtain good and reproducible results, usage of sterile reagents and clean materials is strongly recommended. All basic reagents such as washing and dilution buffers, water, must be devoid of contamination.

To ensure pH stability, incubation at 37°C should be performed in a humidified atmosphere of 5% CO_2

Problems	Possible Sources	Solutions
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Inappropriate storage	Aliquot standard and store at -70°C
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature or timing	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples