Version 15-04/24

User's Manual

IL17 Mouse ELISA Kit

REF **DEIA1519**

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

For the quantitative measurement of Mouse IL17 concentrations in serum, plasma and cell culture supernatants.

General Description

The protein encoded by this gene is a proinflammatory cytokine produced by activated T cells. This cytokine regulates the activities of NF-kappaB and mitogen-activated protein kinases. This cytokine can stimulate the expression of IL6 and cyclooxygenase-2 (PTGS2/COX-2), as well as enhance the production of nitric oxide (NO). High levels of this cytokine are associated with several chronic inflammatory diseases including rheumatoid arthritis, psoriasis and multiple sclerosis.

Principles of Testing

The Mouse IL-17 ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of mouse IL-17 in serum, plasma and cell culture supernatants. This assay employs an antibody specific for mouse IL-17 coated on a 96-well plate. Standards and samples are pipetted into the wells and IL-17 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse IL17 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IL-17 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents And Materials Provided

1. IL-17A Microplate (Item A), 96 wells (12 strips x 8 wells) coated with anti-Mouse IL-17A
2. Wash Buffer Concentrate (20×) (Item B), 25 ml of 20× concentrated solution.
3. Standard Protein (Item C), 2 vials of Mouse IL-17A. 1 vial is enough to run each standard in duplicate.
4. Detection Antibody IL-17A (Item F), 2 vials of biotinylated anti-Mouse IL-17A. Each vial is enough to assay half the microplate.
5. HRP-Streptavidin Concentrate (Item G), 200 µl 700× concentrated HRP-conjugated streptavidin.
6. TMB One-Step Substrate Reagent (Item H), 12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution.
7. Stop Solution (Item I) 8 ml of 0.2 M sulfuric acid.
8. Assay Diluent A (Item D), 30 ml of diluent buffer, 0.09% sodium azide as preservative.
9. Assay Diluent B (Item E), 15 ml of 5× concentrated buffer.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm.

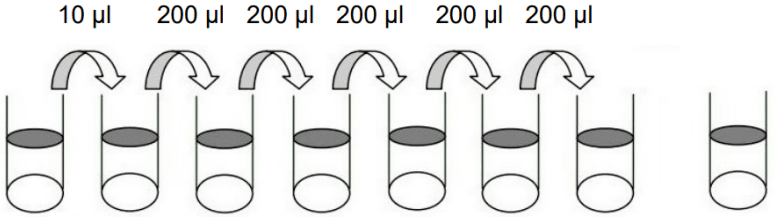
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

Storage

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

Reagent Preparation

1. Bring all reagents and samples to room temperature (18-25°C) before use.
2. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use.
3. Sample dilution: Assay Diluent A (Item D) should be used for dilution of serum and plasma samples. 1X Assay Diluent B (Item E) should be used for dilution of cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2-5 fold. **Note: Levels of IL-17A may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.**
4. Preparation of standard: Briefly spin a vial of Item C. Add 400 µl Assay Diluent A (for serum/plasma samples) or 1× Assay Diluent B (for cell culture medium) into Item C vial to prepare a 50 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Add 10 µl IL-17 standard from the vial of Item C, into a tube with 823.3 µl Assay Diluent A or 1× Assay Diluent B to prepare a 600 pg/ml stock standard solution. Pipette 300 µl Assay Diluent A or 1× Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1× Assay Diluent B serves as the zero standard (0 pg/ml).



		Std1	Std2	Std3	Std4	Std5	Std6	Zero Standard
Diluent volume	Item C + 400 µl	823.3 µl	300 µl	300 µl	300 µl	300 µl	300 µl	300 µl
Conc.	50 ng/ml	600 pg/ml	240 pg/ml	96 pg/ml	38.4 pg/ml	15.4 pg/ml	6.1 pg/ml	0 pg/ml

5. If the Wash Concentrate (20×) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1× Wash Buffer.
6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1× Assay Diluent B (Item E) into

the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1× Assay Diluent B (Item E) and used in step 5 of Part VI Assay Procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 700-fold with 1× Assay Diluent B (Item E). For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 20 µl of HRP-Streptavidin concentrate into a tube with 14 ml 1× Assay Diluent B to prepare a 700-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Assay Procedure

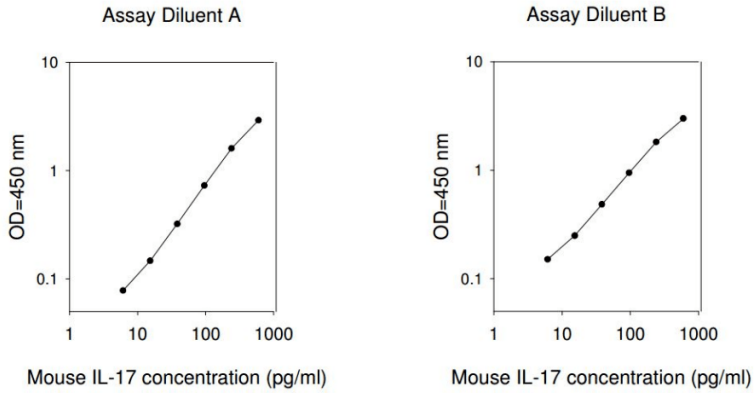
1. Bring all reagents and samples to room temperature (18-25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µl of each standard (see **Reagent Preparation step 3**) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.
4. Discard the solution and wash 4 times with 1× Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µl of 1× prepared biotinylated antibody (**Reagent Preparation step 6**) to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µl of prepared Streptavidin solution (see **Reagent Preparation step 7**) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Calculation

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Standard Curve

These standard curves are for demonstration only. A standard curve must be run with each assay.



Precision

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

Sensitivity

The minimum detectable dose of Mouse IL-17A was determined to be 6 pg/ml. Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Specificity

This ELISA kit shows no cross-reactivity with any of the cytokines tested: Mouse CD30, L CD30, T CD40, CRG-2, CTACK, CXCL16, Eotaxin , Eotaxin-2, Fas Ligand, Fractalkine, GCSF, GM-CFS, IFN-gamma, IGFBP-3, IGFBP-5, IGFBP-6, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-3 Rb, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, KC, Leptin R, Leptin (OB), LIX, L-Selectin, Lymphotactin, MCP-1, MCP-5, M-CSF, MIG, MIP-1 alpha, MIP-1 gamma, MIP-2, MIP-3 beta, MIP-3 alpha, PF-4, P-Selectin, RANTES, SCF, SDF-1 alpha, TARC, TCA-3, TECK, TIMP-1, TNF-alpha, TNF RI, TNF RII, TPO, VCAM-1, VEGF.

Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	94	97	98
	Range (%)	84-103	85-105	86-105
1:4	Average % of Expected	97	98	101.2
	Range (%)	85-104	86-105	87-107

Recovery

Recovery was determined by spiking various levels of Mouse IL-17A into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	101.38	87-107
Plasma	97.47	84-103
Cell culture media	99.62	85-105

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