Science for Society

Mouse Interleukin-17A (IL-17A) ELISA Kit

to determine Mouse Interleukin-17A in Serum, Blood Plasma, Saliva, Urine,

And Other Related Tissue Liquid Samples.

INSTRUCTION MANUAL

FOR ELISA KIT No: LToM3411EA

Arsh Biotech Pvt. Ltd.

308, Aggarwal City Mall, Road No.44,
Pitampura, Delhi-110034, India
Mobile: +91-98105-21400 | Fax: +91-11-42208444
info@arshbiotech.com

INTENDED USE

This kit is used to assay the Mouse Interleukin-17A (IL-17A) in the sample of serum, blood plasma, saliva, urine, and other related biological liquid. For *in vitro* use only.

MANUAL VERSION 1.02

Science for Society

PRINCIPLE

This kit uses enzyme-linked immune sorbent assay (ELISA) based on the double antibody sandwich technology to assay the Mouse Interleukin-17A (IL-17A). An antibody specific for IL-17A has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-17A present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IL-17A is added to the wells. After washing, Streptavidin-HRP conjugate is added to the wells. Following a wash to remove any unbound streptavidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-17A bound in the initial step. The color development is stopped and the intensity of the color is measured.

MATERIALS SUPPLIED IN THIS KIT

Mouse IL-17A microplate: 96 well polystyrene microplates (12 strips of 8 wells) coated with the antibody specific for Mouse IL-17A.

Mouse IL-17A standard: Mouse IL-17A in a buffered protein base with preservatives;lyophilized.

Sample diluent: Diluent solution for reconstituted standard and samples. 5X liquid.

Mouse IL-17A detection antibody: Biotin-conjugated Mouse IL-17A detection antibody. 100X liquid.

Streptavidin-HRP: 100X liquid.

Assay buffer: Diluent solution for detection antibodies and Streptavidin-HRP. 5X liquid.

HRP substrate: TMB (Tetramethyl-benzidine) solution.

Stop solution: 2N sulfuric acid.

Wash buffer: PBS with 1% Tween-20; 20× liquid.

Plate covers.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips
4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

IMPORTANT NOTES

1. Before using, keep the kit outside and allow it to come to room temperature.
2. After breaking the seal of ELISA coated-plate, keep the unused strips in the zipper bag at 2-8 °C.
3. Pipette tips and seal plate membrane should not be used more than once in order to avoid cross contamination.
4. All samples and all discard generated should be disposed as per local rules.
5. Reagents of different batches must not be mixed and should be used before their respective validity dates.
6. Substrate is sensitive to light and therefore should not be exposed to light for too long.

PRECISION

Intra-assay Precision (Precision within an assay): Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%) <10%.

Inter-assay Precision (Precision between assays): Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%) <10%.

Recovery: The recovery of Mouse IL-17A spiked to different levels in samples throughout the range of the assay in various matrices was evaluated. The recovery ranged from 98% to 116% with an overall mean recovery of 106%

Sensitivity: The minimum detectable dose (MDD) of Mouse IL-17A is typically less than 4 pg/ml. The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity: Mouse IL-17A ELISA Kit can be used to measure natural and recombinant Mouse IL-17A. Mouse IL-17A ELISA Kit has high sensitivity and excellent specificity for detection of Mouse IL-17A. No significant cross-reactivity or interference between Mouse IL-17A and analogues was observed.

SAMPLE PREPARATION

1. Samples containing NaN_3 are not recommended for testing with ELISA as they may inhibit the activity of Horse Radish Peroxidase (HRP).
2. After extraction, experiment should be conducted immediately. Otherwise, keep the sample at -20 °C. Avoid repeated freeze-thaw cycles.
3. Serum: Allow the sample to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RCF) for 20 minutes. Collect the supernatant carefully. In case of storage, centrifugation should be performed again prior to use.
4. Blood plasma: During sample collection EDTA or sodium citrate should be used for anti-coagulation. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.

5. Urine: Collect the sample in a sterile tube. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow a similar procedure.

6. Cell culture supernatant: For secreted components, centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells by repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.

7. Tissue sample: Incise tissue sample of interest and add few mls of PBS (pH 7.4). Freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8 °C. Add few mls of PBS (pH 7.4) and then homogenize the sample thoroughly by hand or by homogenizer. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use.

REAGENT PREPARATION

Mouse IL-17A detection antibody - Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated detect antibody solution with Assay buffer in a clean plastic tube as needed according to the standards and samples. Detect antibody should be used within 30 minutes after dilution.

Streptavidin-HRP - Mix well prior to making dilutions. Make a 1:100 dilution of the concentrated Streptavidin-HRP with Assay buffer in a clean plastic tube as needed according to the standards and samples. Streptavidin-HRP should be used

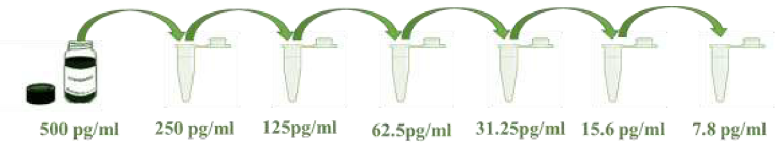
within 30 minutes after dilution.

Sample dilution - If your samples need to be diluted, Sample diluent is used for dilution of serum/plasma samples, while cell culture medium is used for dilution of cell culture supernates.

ASSAY PROCEDURE

1. Dilution of standard solutions: This kit contains a standard of known concentration, which could be diluted in small tubes by the end-user by following the instruction in the table below:

250pg/ml	Standard No.6	500µl Original Standard + 500µl Standard diluents
125pg/ml	Standard No.5	500µl Standard No.5 + 500µl Standard diluents
62.5pg/ml	Standard No.4	500µl Standard No.4 + 500µl Standard diluent
31.25pg/ml	Standard No.3	500µl Standard No.3 + 500µl Standard diluent
15.6pg/ml	Standard No.2	500µl Standard No.2 + 500µl Standard diluent
7.8pg/ml	Standard No.1	500µl Standard No.1 + 500µl Standard diluent



Prepare all reagents and working standards as directed in the previous sections.

2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.

3. Add 100 µl of diluted standard and sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided for a record of standards and samples assayed.

4. Aspirate each well and wash, repeating the process twice for a total of three washes. Wash by filling each well with Wash buffer (250 µl) using a squirt bottle,

multi-channel pipette, manifold dispenser, or automatic washer.

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 diluted Mouse IL-17A detection antibody to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.

6. Repeat the aspiration/wash as in step 4.

7. Add 100 µl of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 30 minutes at room temperature. Avoid placing the plate in direct light.

8. Repeat the aspiration/wash process for five times as in step 4.

9. Add 100 µl of HRP substrate solution to each well. Incubate for 15 minutes at room temperature. Protect from light.

10. Add 50 µl of Stop solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

PROTOCOL SUMMARY

Prepare reagents, samples and standards.

↓

Add 100 µl samples and standards in respective wells and incubate for 2 hours at RT.

↓

Wash the plate three times. Add 100 µl of detection antibody and incubate for 1 hour at RT.

↓

Wash the plate three times. Add µl of diluted streptavidin-HRP to each well, incubate for 30 minutes at RT, and wash plate five times.

↓

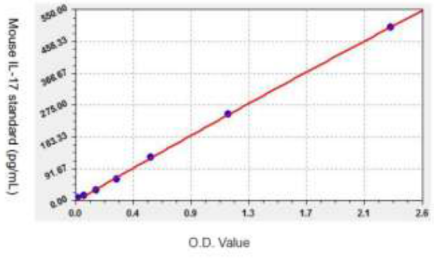
Add 100 µl substrate solution and incubate for 15 minutes at RT.

↓

Add 50 µl stop solution to each well, measure O.D. at 450nm within 30 min.

↓

Plot and Calculate.



Standard IL-17 (pg/mL)	Optical Density (450nm)	Average	Corrected
0	0.028	0.049	—
	0.070		
7.8	0.070	0.069	0.02
	0.068		
15.6	0.109	0.115	0.066
	0.124		
31.25	0.207	0.206	0.157
	0.205		
62.5	0.346	0.353	0.304
	0.360		
125	0.606	0.6065	0.5575
	0.607		
250	1.151	1.182	1.133
	1.213		
500	2.321	2.3835	2.3345
	2.446		

THE CHART SHOWN HERE IS FOR REFERENCE PURPOSES ONLY AND THE ACTUAL PERFORMANCE MAY VARY.

CALCULATION

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration for each standard on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Mouse IL-17A concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

ASSAY RANGE： 7.8 pg/ml – 500 pg/ml .

PACKAGE SIZE： 96Tests.

SENSITIVITY： <4pg/ml.

Linearity: To assess linearity of the assay, samples containing and/or spiked with high concentrations of Mouse IL-17A were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

VALIDITY & STORAGE: One year (at 2-8°C).