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Mouse IL-17A Antigen ELISA Kit

Catalog # MIL17AKT

Strip well format. Reagents for up to 96 tests.

Rev: Mar 2014

INTENDED USE

This mouse IL-17A antigen assay is intended for the quantitative determination of total mouse IL-17A antigen in cell culture media. **For research use only.**

BACKGROUND

Mouse Interleukin-17A (aka IL-17, IL-17A or CTLA-8) is a 133 amino acid disulfide-linked homodimeric glycoprotein that is the founding member of the IL-17 family of proteins [1-2]. IL-17A is a proinflammatory cytokine that participates in neutrophil recruitment and is primarily expressed in CD4+ T cells [3]. IL-17A has been shown in a mouse knockout model to play a vital role in allergen-specific immune responses via T cell activation [4]. It binds to the IL-17RA and IL-17RC receptors which are expressed in TH17, CD8+ T, γδT, NK, NKT and LTi cells [5].

ASSAY PRINCIPLE

Mouse IL-17A will bind to the capture antibody coated on the microtiter plate. After appropriate washing steps, biotinylated primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with horseradish peroxidase conjugated streptavidin. Following an additional washing step, TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of mouse IL-17A. The amount of color development is proportional to the concentration of mouse IL-17A in the samples.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-mouse IL-17A antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Mouse IL-17A standard:** 1 vial lyophilized standard
- **Anti-mouse IL-17A primary antibody:** 1 vial lyophilized polyclonal antibody
- **Horseradish peroxidase-conjugated Streptavidin:** 1 vial concentrated HRP labeled streptavidin
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 100ng/ml standard solution.

Dilution table for preparation of mouse IL-17A standard:

IL-17A concentration (ng/ml)	Dilutions
10	900µl (BB) + 100µl (from vial)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.2	600µl (BB) + 400µl (0.5ng/ml)
0.1	500µl (BB) + 500µl (0.2ng/ml)
0.05	500µl (BB) + 500µl (0.1ng/ml)
0.02	600µl (BB) + 400µl (0.05ng/ml)
0.01	500µl (BB) + 500µl (0.02ng/ml)
0	500µl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100µl IL-17A standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures mouse IL-17A antigen in the 0.01-10 ng/ml range. If the unknown is thought to have high IL-17A levels, dilutions may be made in culture media or in blocking buffer.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Streptavidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 1µl of HRP conjugated streptavidin into 5ml blocking buffer to generate a 1:5,000 dilution. Add 2ml of 1:5,000 dilution to 8ml of blocking buffer to generate a 1:25,000 dilution. Add 100µl of the 1:25,000 dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

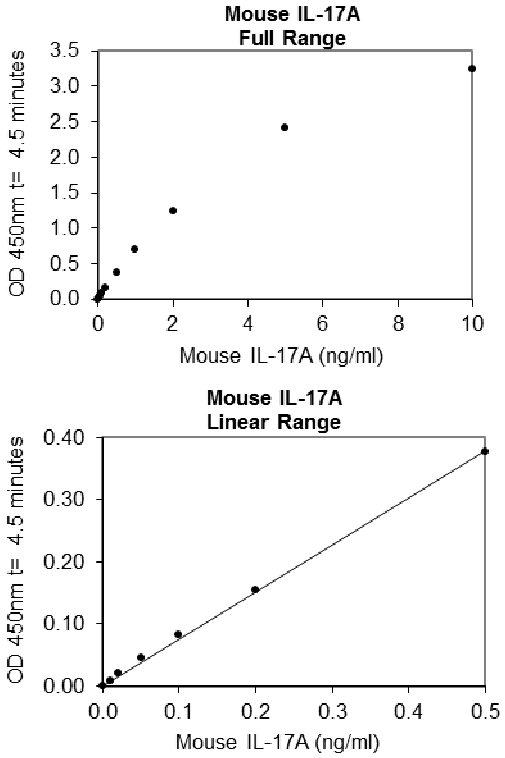
Measurement

Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of IL-17A in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four parameter logistic (4PL) curve fit. The amount of IL-17A in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):



EXPECTED VALUES

IL-17A in normal human serum has been reported to be 7.4 pg/ml in adults [6] and 0.77 pg/ml in children [7]. IL-17A in normal mouse plasma was below the lowest standard, 10 pg/ml. IL-17A in cell culture supernates will vary by cell type, media and culture time.

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.056-0.069) and calculating the corresponding concentration. The MDD was 4.8 pg/ml.

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	0.176	1.82	6.69
Standard Deviation	0.005	0.059	0.196
CV (%)	2.76	3.26	2.93

Inter-assay Precision: Three samples of known concentration were tested in ten independent assays to assess inter-assay precision.

Sample	1	2	3
n	10	10	10
Mean (ng/ml)	0.127	1.90	6.21
Standard Deviation	0.016	0.111	0.376
CV (%)	12.5	5.82	6.06

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in culture media was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/ml)	0.108	0.524	2.02	5.64
Average % Recovery	108	105	101	113
Range	106-112%	101-109%	97-104%	95-123%

Linearity: To assess the linearity of the assay, cell culture samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	92	98	90	91
Range	77-101%	94-101%	87-92%	85-96%

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. Yao Z, *et al.*: J Immunol. 1995, 155:5483-5486.
2. Kolls JK and Lindén A: Immunity. 2004, 21:467-476.
3. Kawaguchi M, *et al.*: J Allergy Clin Immunol. 2004, 114:1265-1273.
4. Nakae S, *et al.*: Immunity. 2002, 17:375-387.
5. Gaffen SL: Nature Reviews Immunology. 2009, 9:556-567.
6. Arican O, *et al.*: Mediators Inflamm. 2005, 5:273-279.
7. Al-Ayadhi LY and Mostafa GA: J Neuroinflammation. 2012, 9:158.

Example of ELISA Plate Layout
96 Well Plate: 22 Standard wells, 74 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.01 ng/ml	0.02 ng/ml	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	
B	0	0.01 ng/ml	0.02 ng/ml	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml	
C												
D												
E												
F												
G												
H												