cellsciences.com

Mouse IL-17A Total Antigen Assay ELISA Kit

Strip well format. Reagents for up to 96 tests

Catalog No.	**CS451A** **CS451B**	**Quantity:**	**1 X 96 tests** **5 x 96 tests**

Intended Use: This mouse IL-17A antigen assay is intended for the quantitative determination of total mouse IL-17A antigen in cell culture media.

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. IL-17A is a proinflammatory cytokine that participates in neutrophil recruitment and is primarily expressed in CD4+ T cells. IL-17A has been shown in a mouse knockout model to play a vital role in allergen-specific immune responses via T cell activation. It binds to the IL-17RA and IL-17RC receptors which are expressed in TH17, CD8+ T, γδT, NK, NKT and LTi cells.

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 450 nm. 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 microtiter strip plate:**
  8X12 removable well strips containing anti-mouse IL-17A antibody on the surface. Strips are blocked and dried.
- **10X Wash Buffer:**
  1 bottle of 50 ml; bring to 1x using DI water
- **Mouse IL-17A standard:**
  1 vial of lyophilized standard
- **Anti-mouse IL-17A primary antibody:**
  1 vial of lyophilized antibody
- **HRP conjugated streptavidin:**
  1 vial concentrated HRP labeled streptavidin.
- **TMB substrate solution:**
  1 bottle of 10 ml solution

Storage and Stability: All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

Reagents and Equipment Required:

- 1-channel pipettes covering 20-200 µl, 200-1000 µl and 500-5000µl
- 12-channel pipette for 30-300µl
- Paper towels or kimwipes

Cell Sciences®
Neponset Valley Tech Park
480 Neponset St., Bldg. 12A
Canton, MA 02021

Toll Free: 888 769-1246
Phone: 781 828-0610
Fax: 781 828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

- 1.5 ml micro centrifuge tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- TBS buffer
- Blocking buffer
- Microtiter plate spectrophotometer operable at 450 nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm

Warnings:

Warning – Avoid skin and eye contact when using TMB substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

Precautions:

- **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.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

Preparation of Reagents:

- **TBS buffer:** 0.1 M Tris, 0.15 M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA in TBS

Sample Preparation:

The assay measures mouse IL-17A in the 0.01-10ng/ml range. Samples giving mouse IL-17A levels above 10ng/ml should be diluted in culture media or blocking buffer before use. Samples of cell culture media may be applied directly to the plate.

Assay Procedure:

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

Preparation of Standard: Reconstitute 100 ng standard vial with 1.0 ml of blocking buffer to give a 100 ng/ml stock solution.

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

Cell Sciences®
Neponset Valley Tech Park
480 Neponset St., Bldg. 12A
Canton, MA 02021

Toll Free: 888 769-1246
Phone: 781 828-0610
Fax: 781 828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

IL-17A concentration (ng/ml)	Dilutions
10	900µl (BB) + 100µl (100ng/ml)
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. Add 100 µl standards in duplicate and unknowns wells. Carefully record position of standards and unknowns. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

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

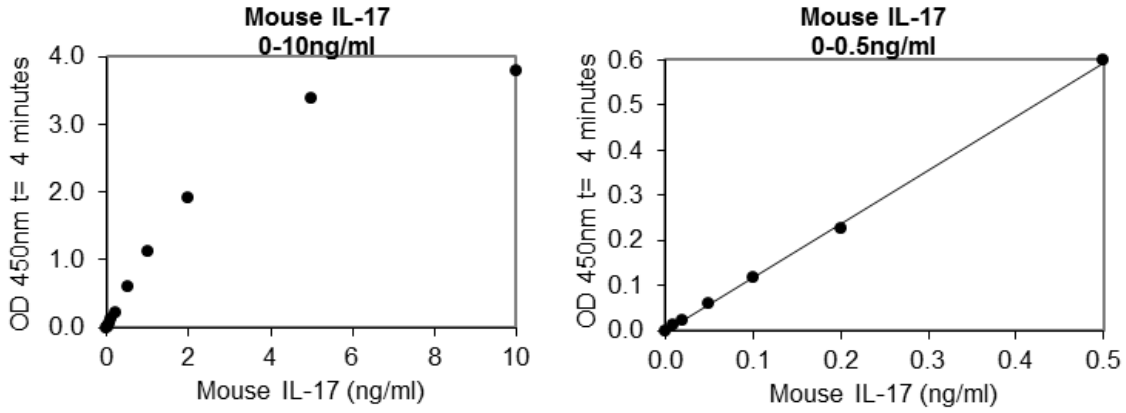
Streptavidin-HRP Addition:
Dilute 1 µl of concentrated streptavidin-HRP conjugate into 5 ml of blocking buffer and mix well. Add 1 ml of the diluted secondary into 9 ml of blocking buffer (for a final dilution of 1:50,000) and add 100 µl to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation:
Add 100 µl 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 50 µl of 1N H_2SO_4 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 and read final absorbance values at 450 nm. For best results, read plate immediately

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

Assay Calibration:
Plot A_{450} against the amount of IL-17A in the standards. Fit a straight line through the linear points of the standard curve points 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 mouse 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, DO NOT USE)



Expected Values: IL-17A in normal human serum has been reported to be 7.4 pg/ml in adults and 0.77 pg/ml in children. 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.

Cell Sciences®
Neponset Valley Tech Park
480 Neponset St., Bldg. 12A
Canton, MA 02021

Toll Free: 888 769-1246
Phone: 781 828-0610
Fax: 781 828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

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 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.

	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.

	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 blocking buffer 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, human plasma 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%

Cell Sciences®
Neponset Valley Tech Park
480 Neponset St., Bldg. 12A
Canton, MA 02021

Toll Free: 888 769-1246
Phone: 781 828-0610
Fax: 781 828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

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 or from contact with the above product.

Plate Layout
96 Well Plate

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

Standards: 20 wells
Samples: 76 wells

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

Cell Sciences®
Neponset Valley Tech Park
480 Neponset St., Bldg. 12A
Canton, MA 02021

Toll Free: 888 769-1246
Phone: 781 828-0610
Fax: 781 828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com