



## Mouse IL-17A ELISA kit (Pre-coated)

**CL89122K-48/ CL89122K-96**

**Lot:**

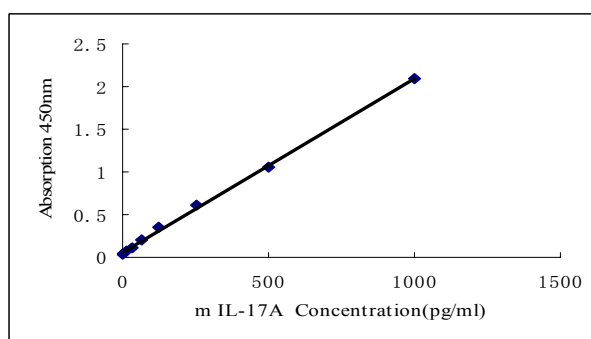
### Product Description:

The Mouse IL-17A ELISA kit is to be used for the in-vitro quantitative determination of IL-17A in mouse serum, plasma, buffered solutions or cell culture medium. The assay will recognize both natural and recombinant mouse IL-17A. This kit has been configured for research use only and is not to be used in diagnostic or therapeutic procedures. Read the manual and check the reagents in the kit before use.

**Standard Curve Range:** 15.6-1000pg/mL

**Sensitivity:** 8pg/mL

**Reproducibility:** Intra-assay CV: < 10%; Inter-assay CV: < 10%



**Note: Typical IL-17A standard curve ranging from 15.6 to 1000pg/mL**

### Reagents Provided and Reconstitution:

Reagents	Quantity	Reconstitution
Cytokine standard	2/1 vial*	Lyophilized, reconstitute with the volume of reagent indicated on the vial
Biotinylated antibody	2/1 vial *	Dilute 100 times in Diluent buffer, 1x
Streptavidin-HRP	2/1 vial *	Dilute 100 times in Diluent buffer, 1x
Diluent buffer, 1x	3/2 vial *	Ready-to-use
Washing buffer, 50x	1 vial	Dilute 50 times in distilled Water
TMB	1 vial	Ready-to-use
Stop solution	1 vial	Ready-to-use
Pre-coated ELISA plates	8×12 or 8×6*	Ready-to-use
Plastic cover	2/1 sheet *	Ready-to-use

**\*: 96 /48 Tests**



### **Material Required but not Provided:**

1. 96-well ELISA plate reader (microplate spectrophotometer).
2. Pipettes and pipette tips: 10  $\mu$ L, 50  $\mu$ L, 100  $\mu$ L, 200  $\mu$ L and 1000  $\mu$ L.
3. Distilled water, fresh absorbent paper.
4. Vortex mixer and magnetic stirrer

### **Protocol Notes/ Lab. Quality Control:**

1. When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels. All reagents should be warmed to room temperature before use. Lyophilized standards should be discarded after use.
2. Centrifuge Streptavidin-HRP and Biotinylated antibody immediately before use.
3. Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from deterioration. Cover or cap all reagents when not in use.
4. Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross-contamination.
5. Thoroughly mix the reagents and samples before use by agitation or swirling.
6. When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
7. Use a clean plastic container to prepare the washing solution.
8. All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
9. The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. Warning TMB is toxic avoid direct contact with hands. Dispose of TMB when it turns blue. Please read absorbance within 10min after adding the stop solution.
10. Respect incubation times and temperature described in the assay procedure.
11. Do not use reagents beyond the expiration date of the kit. Do not mix or interchange reagents between different lots.
12. Do not extrapolate the standard curve range. The dose-response is non-linear out of the range and good accuracy is difficult to obtain.
13. Specificity: No cross-reactivity to other human cytokines.

### **Preparation of Reagents:**

1. **Cytokine standard:** First reconstitute the freeze-dried material with the volume of reagent indicated on the vial. Stand for 5 minutes and mix gently to ensure complete solubilisation. Dilute the standard to recommending concentration with Diluent buffer, 1x .  
**Serial dilution of standard to 1000pg/mL, 500pg/mL, 250pg/mL, 125pg/mL, 62.5 pg/mL, 31.3pg/mL, 15.6pg/mL is recommended.**
2. **Biotinylated antibody:** Dilute 100 times with Diluent buffer, 1x, in a clean glass vial as needed according to the following table:

Number of Wells used	Biotinylated Antibody ( $\mu$ L)	Diluent buffer, 1x ( $\mu$ L)
16	8.5	850
24	13	1300
32	17	1700
48	25	2500
96	50	5000

3. **Streptavidin-HRP:** Dilute 100 times with Diluent buffer, 1x, in a clean glass vial as needed according to the following table:

Number of Wells	Streptavidin-HRP ( $\mu\text{L}$ )	Diluent buffer, 1x (mL)
16	17	1.7
24	25	2.5
32	34	3.4
48	50	5
96	100	10

4. **Washing buffer, 50x:** Dilute 50 times in distilled water.

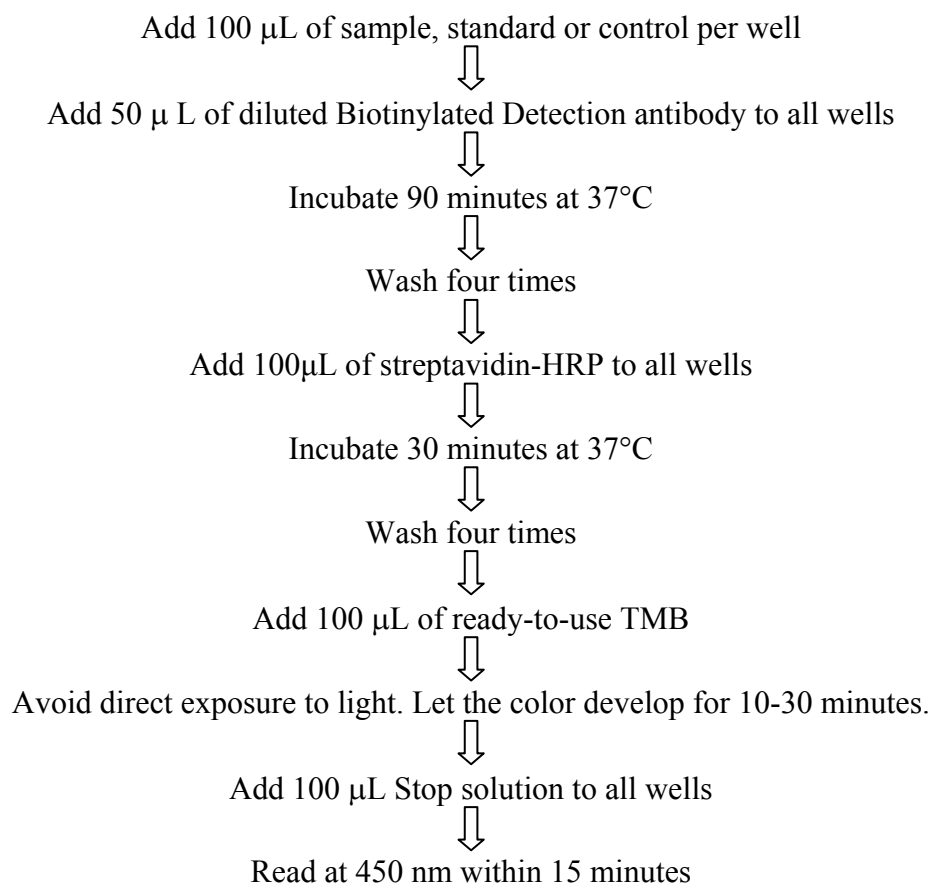
**Assay Method:**

1. Before use, mix all reagents thoroughly without making foam.
2. Determine the number of microwell strips required to test the desired number of samples, plus appropriate number of wells needed for running blanks and standards. Each sample, standard and blank should be assayed in duplicate. Remove sufficient microwell strips from the pouch.
3. Add 100  $\mu\text{L}$  of diluted Cytokine standard to standard wells, 100  $\mu\text{L}$  of sample to sample wells, 100  $\mu\text{L}$  of standard diluent to the blank wells.
4. Add 50  $\mu\text{L}$  of diluted Biotinylated antibody to all wells. Cover with a plate cover and incubate 90 minutes at 37°C.
5. Remove the cover and wash the plate as follows: Aspirate the wells to remove liquid and wash the plate 4 times using 300 $\mu\text{L}$  of wash buffer per well. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess wash buffer. Use the microwell strips immediately after washing.
6. Add 100  $\mu\text{L}$  of diluted streptavidin-HRP to all wells. Cover and incubate 30 minutes at 37°C.
7. Remove the cover and empty wells. Wash microwell strips according to step 5. Proceed immediately to the next step.
8. Pipette 100  $\mu\text{L}$  of TMB substrate solution into all wells, including the blank wells and incubate in the dark for 10-30 minutes at 37°C. Avoid direct exposure to light by wrapping the plate in aluminum foil.
9. The enzyme-substrate reaction is stopped by quickly pipetting 100  $\mu\text{L}$  of Stop solution into each well.
10. Measure the optical density in an ELISA reader at 450 nm (optionally 610-650 nm as the reference wave length) within 15 minutes. Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of the samples and the standards.

**Data Analysis:**

- a) Generate a standard curve by reducing the data using computer software capable of generation 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 on the x-axis and draw a best fit curve through the points on the graph.
- b) If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

**Assay Procedure Summary:**



## **Appendix:**

<b>ELISA Troubleshooting Guide</b>		
<b>Problem</b>	<b>Possibility</b>	<b>Solution</b>
<b>1.</b> Very weak signal	(1) Improper and inefficient washing	(1) correct Washing
	(2) Reagents have expired	(2) Do not use the expired reagents
	(3) Incorrect dilutions, conjugate concentration was too low	(3) Repeat test using correct dilutions, check with the recommendations of the antibody manufacturer
	(4) Inefficient incubation time	(4) Repeat ELISA, follow the protocol carefully for each step's incubation time
	(5) Incorrect storage of reagents	(5) Store reagents in the correct temperature, avoid freeze and thaw, avoid using the "frost free" freezer
	(6) Wrong filter in ELISA reader was used	(6) Use the correct wavelength setting
<b>2.</b> Poor standard curve and duplicates	(1) Improper and inefficient washing	(1) correct Washing
	(2) Poor mixing of samples	(2) Mix samples and reagents gently and warm to room temperature
	(3) Plates not clean	(3) Plates should be wiped on bottom before measuring absorbance
	(4) Wrong filter in ELISA reader was used	(4) Use the correct wavelength setting
	(5) Mix or cross-use reagents between different lots	(5) Use reagents from the same kit
	(6) Cross-contamination between wells	(6) Use a clean disposable plastic pipette tip for each reagent, standard or sample addition in order to avoid cross-contamination
	(7) Samples and reagents were absorbed to the uncoated district	(7) Adding the liquid by gently lowering an aspiration tip into the center of each well
	(8) Inconsistent incubation, washing and color development time	(8) Use a timer
<b>3.</b> No signal	(1) Enzyme inhibitor present in your system e.g., sodium azide in the washing buffer and diluent buffer inhibits peroxidase activity	(1) Repeat ELISA, make sure your system contains no enzyme inhibitor
	(2) Wrong reagents were used or some steps have been omitted	(2) Repeat ELISA, follow the protocol carefully and use the correct reagents
	(3) Reagents have expired	(3) Do not use the expired reagents
<b>4.</b> High background	(1) Improper and inefficient washing	(1) correct Washing
	(2) Contaminated substrate	(2) Substrate should be colorless
	(3) Reagents have expired	(3) Do not use the expired reagents
	(4) Incorrect dilutions, e.g., conjugate concentration was too high	(4) Repeat test using correct dilutions, check with the recommendations of the antibody manufacture
	(5) Distilled water contaminated by HRP et al.	(5) Use fresh distilled water
	(6) Cross contamination with other positive samples	(6) Repeat ELISA, be careful when washing and pipetting
	(7) Incubator temperature over 37°C or reaction time is too long	(7) Calibrate the incubator temperature; shorten the color development time

**Laboratory Reagent For Research Use Only**