Mouse IgE Precoated ELISA Kit

Cat#:1218202

[Product Description]

The Mouse IgE Precoated ELISA Kit is used for the in vitro quantitative detection of mouse IgE in mouse serum, plasma, buffer or cell culture medium. It can detect both natural and recombinant mouse IgE. This kit is only used for scientific research. It shall not be used for diagnosis. Read the instruction and check the components of the kit before use. If there are any questions, please contact with Shenzhen Dakewe Bio-engineering Co., Ltd.

Detection range: 20~0.313 ng/mL

Sensitivity: 80 pg/mL

Precision: Intra-Assay CV: ≤10%; Inter-Assay CV: ≤15%

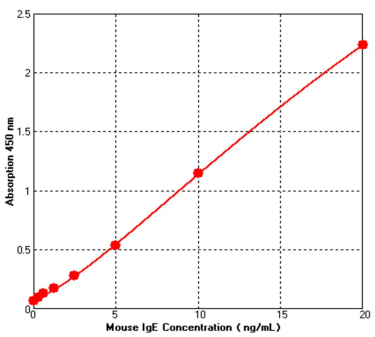

*Figure 1.*Note: Mouse IgE standard curve range: 20~0.313 ng/mL

[Materials Provided]

Product Name	Cat No.	Component Name	Size	Quantity	Reconstitution
Mouse IgE Precoated ELISA Kit	1218202	Cytokine Standard	48 T	2 vials	Lyophilized, diluted according to the instructions on the vial
		Biotinylated Antibody	50 μL	2 vials	1:100 diluted with Dilution Buffer R (1×)
		Streptavidin-HRP	50 μL	2 vials	1:100 diluted with Dilution Buffer R (1×)
		Dilution Buffer R(1×)	25 mL	3 bottles	Ready-to-use
		Washing Buffer(50×)	15 mL	1 bottle	1:50 diluted with distilled water
		TMB	10 mL	1 bottle	Ready-to-use
		Stop Solution	10 mL	1 bottle	Ready-to-use
		Precoated 96-well strip ELISA plates	96 T	1 plate	Ready-to-use
		Plate Sealers	/	4 strips	Ready-to-use

[Self-provided Items]

1. Microtiter plate reader fitted with appropriate filters (450 nm required with optional 620 nm reference filter）.
2. Adjustable Pipettes and pipette tips.
3. Distilled or deionized water, brand new filter paper.
4. Vortex mixer and magnetic stirrer.

[Cautions]

1. When the kit is not in use, it shall be stored according to the label on the vial or bottle. Pre-warmed to room temperature (18℃~25℃) before use. The lyophilized standards shall be discarded after use.
2. Streptavidin-HRP and Biotinylated Antibody shall be centrifuged briefly before use.
3. The plate strips not used in the experiment shall be immediately put back into the package and sealed to prevent contamination. The reagents shall be packaged or covered when not in use.
4. When adding reagents, standards, and samples in experimental operations, please use disposable

tips to avoid cross-contamination.

5. The solution or reagent is mixed evenly by shaking or stirring.
6. The order of adding reagents to the wells of the experimental plate shall be consistent to ensure the same incubation time for all reaction wells.
7. Clean plastic containers are used for washing liquid.
8. In the process of washing, the washing liquid is removed by suction or shaking off, and then fully patted dry on the filter paper. Do not put the filter paper directly into the reaction well to absorb water.
9. TMB is sensitive to light, so long time exposure to light shall be avoided, and metal contact shall be prevented from affecting the results. If the TMB to be used turns blue, it indicates that the TMB has been contaminated, so please discard it. Please read the OD value within 10 minutes after stopping the reaction.
10. Please incubate strictly according to the time and temperature indicated in the instructions.
11. Do not use expired kits, and do not mix reagents from different lots.
12. Do not exceed the linear range. The results beyond the linear range are non-linear, and accurate results cannot be obtained according to this standard curve.
13. Specificity: Does not react with other mouse cytokines.

[Preparation of Reagents]

1. **Cytokine Standard:** The lyophilized powder is resuspended according to the label instructions; let it stand for 5 minutes, and shake it slightly until it is completely dissolved. Then it is diluted with Dilution Buffer R(1×) to 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, 0.313 ng/mL.

   Dilution step: Dissolve the Cytokine Standard according to the bottle label and transfer it into an EP tube, labeled as C7. Take another 6 EP tubes, labeled as C6/C5/C4/C3/C2/C1, and add 250 μL of Dilution Buffer R(1×) to each tube. Take 250 μL of Cytokine Standard from C7 and transfer it to C6 tube for 2-fold dilution. Dilute C5/C4/C3/C2/C1 tube in the same way. Only add Dilution Buffer R(1×) to C0 tube.

Standard code	Dilution ratio	Dilution Buffer R(1×) (μL)	Standard Concentration (pg/mL)
C7	---	---	20
C6	1：2	250	10
C5	1：4	250	5
C4	1：8	250	2.5
C3	1：16	250	1.25
C2	1：32	250	0.625
C1	1：64	250	0.313
C0	---	250	0

2. **Biotinylated Antibody:** By referring to the following table, it is diluted with Dilution Buffer R (1×) into a clean tube at the ratio of 1:100, and mixed well to make 1×working solution.

Number of Wells	Biotinylated Antibody (μL)	Dilution Buffer R(1×) (μL)
16	17	1700
24	25	2500
32	34	3400
48	50	5000
96	100	10000

3. **Streptavidin-HRP:** By referring to the following table, it is diluted with Dilution Buffer R (1×) into a clean tube at the ratio of 1:100, and mixed well to make 1×working solution.

Number of Wells	Streptavidin-HRP (μL)	Dilution Buffer R(1×) (μL)
16	17	1700
24	25	2500
32	34	3400
48	50	5000
96	100	10000

4. **Washing Buffer (50×):** It is diluted with distilled water at 1:50.

[Operation Process]

1. Before use, all reagents are mixed thoroughly to avoid foaming.
2. According to the number of experimental holes (blank and standard), the number of plate strips required is determined. Samples, standards and blanks shall be tested in duplicate well.
3. The 100 μL of diluted Cytokine Standard is added to each standard well. 100 μL of sample is added to each sample well. And 100 μL of Dilution Buffer R (1×) is added to the blank control wells, Covered with the plate sealer and incubated at 37 ℃ for 90 minutes.
4. The liquid in the wells is removed; 300 μL of 1×Washing Buffer is added to each well, and the liquid is discarded after staying for 1 minute. Repeat 4 times. The wells are dried on the filter paper for the last time.
5. The 100 μL of diluted Biotinylated Antibody is added to each well. Covered with the plate sealer and incubated at 37 ℃ for 60 minutes.
6. Repeat Step 4.
7. The 100 μL of diluted Streptavidin HRP is added to each well. Covered with the plate sealer and incubated at 37 ℃, for 30 minutes.
8. Repeat Step 4.
9. The 100 μL of TMB is added to each well. Incubated for 10~20 minutes in dark.
10. The 100 μL of Stop Solution is quickly added to the wells to stop the reaction.
    Within 10 minutes after stopping, the absorbance values are read at 450 nm .If the reader is capable of reading at 620 nm,the absorbance at 620 nm can be subtracted from the absorbance at 450 nm.

[Data Analysis]

A). Logarithm of coordinate or natural number of fitting curve is recommended. The fitting equation is usually a straight line, a quadratic equation and a four-parameter equation. The best standard curve is selected by fitting of various application software, and the corresponding concentration is found according to the OD value of the sample.

B). The diluted sample shall be multiplied by the dilution multiple when calculating the concentration.

[List of Operation Steps]

100 μL of sample and standard are added to each well, and the control is set

⇩

Incubated at 37℃ for 90 minutes and washed for 4 times

⇩

100 μL of diluted Biotinylated Antibody is added to each well

⇩

Incubated at 37℃ for 60 minutes and washed for 4 times

⇩

100 μL of diluted Streptavidin HRP is added to each well

⇩

Incubated at 37℃ for 30 minutes and washed for 4 times

⇩

100 μL of ready-to-use TMB is added to each well and incubated in the dark place for 10~20 minutes

⇩

100 μL of Stop Solution is added to each well

⇩

The plate is read at 450 nm in 10 minutes

[Storage]

The kit is stored at 2℃~8℃ and can be stored stably for 12 months. The kit cannot be used after expiration.

[Appendix]

Troubleshooting Guide		
Problems	**Possible reasons**	**Solutions**
1. Very weak results	(1) Incorrect washing method	(1) The correct method is used to wash the strips
	(2) Reagents have expired	(2) Do not use the expired reagents
	(3) Enzyme conjugate diluent concentration is too low	(3) Repeat the experiment according to the recommended dilution concentration of the manufacturer's instructions
	(4) Inefficient incubation time	(4) Repeat the test according to the incubation time of each step
	(5) Incorrect storage of reagents	(5) Reagents are stored correctly to avoid repeated freezing and thawing
	(6) Wrong filter in microplate reader was used	(6) The correct microplate reader wavelength setting is used
2. The repeatability of standard curve and determination is poor	(1) Incorrect washing method	(1) The correct method is used to wash the strips
	(2) Uneven dilution	(2) Return the reagent to room temperature, and the sample and reagent are gently diluted
	(3) Plates are not clean	(3) The bottom of the plate is wiped clean before detecting the wavelength
	(4) Wrong filter in microplate	(4) The correct wavelength parameters of

	reader was used	microplate reader are set up
	(5) Reagents from different lots are mixed and cross-used	(5) The reagents in the same batch of kits are used
	(6) Cross contamination in the well Inconsistency of incubation time, washing plate and color development time	(6) Disposable suction tips are used to add sample or standard to avoid cross contamination
	(7) Samples and reagents are added to the non-coated area	(7) The liquid is released near the middle of the plate well
	(8) Incorrect washing, incubation and color development time	(8) Time was used to correct the incubation time of each step
3. Blank plate (positive control does not develop color)	(1) There are problems in the preparation of the plate washer, for example, the measuring cylinder is not clean, containing enzyme inhibitors (such as sodium azide), etc	(9) Repeat test, and make sure that the reagent does not contain enzyme inhibitors
	(2) Wrong reagents were added or omitted	(10) Read the instructions carefully and repeat the test with the correct reagents
	(3) Reagents have expired	(11) Do not use the expired reagents
4. Blank background is high	(1) Incorrect washing method	(12) The correct method is used to wash the strips
	(2) The color developing fluid has deteriorated	(13)The color developing solution before use shall be colorless
	(3) Reagents have expired	(14) Do not use the expired reagents
	(4) Incorrect reagent dilution, for example the concentration of enzyme added is too high	(15) Repeat the experiment according to the recommended dilution concentration of the manufacturer
	(5) Distilled water is contaminated by enzymes	(16)Fresh distilled water is used
	(6) Contaminated by other positive samples	(17) When repeating the test, i.e. adding samples and washing, be careful not to cross-contamination
	(7)The temperature of the incubator exceeds 37°C or the reaction time is too long	(18) The color reaction time is appropriately shortened

[Company Information]

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