



COSMO BIO CO., LTD.
Inspiration for Life Science

IgE EIA KIT (Mouse)

Cat No. YMS-7675



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PURPOSE

Determination of mouse IgE in serum.

KIT COMPONENT

1. Anti-Mouse IgE Plate	8wells/12 strips	1set
2. Anti-Mouse IgE Enzyme Conjugate	11mL	1 vial
3. Mouse IgE Standard 10ng/mL	1mL	1 vial
4. Mouse IgE Standard 50ng/mL	1mL	1 vial
5. Mouse IgE Standard 100ng/mL	1mL	1 vial
6. Mouse IgE Standard 250ng/mL	1mL	1 vial
7. Mouse IgE Standard 500ng/mL	1mL	1 vial
8. Sample Diluent, 0ng/mL Standard	30mL	1 bottle
9. Chromogen Substrate	15mL	1 bottle
10. Washing Concentrate	50mL	1 bottle
11. Stopping Solution	12mL	1 bottle

ASSAY PROCEDURE

A. Equipment

Plastic disposable tube
Tube rack
Micropipette or Multi-channel micropipette(10, 100, 300 μ L)
Measuring cylinder
Vortex mixer
Incubator
Refrigerating centrifuge
Aspirator or Microplate washer
Microplate reader

B. Preparation of Reagents

Washing Solution
Add purified water to 50mL of Washing Concentrate to a final volume of 500mL.

C. Preparation of Sample

Sample should be diluted 25 times with prepared Sample Diluent.
(e.g. Add 10 μ L of serum sample to 240 μ L of Sample Diluent)
Results exceeding IgE level of 500 ng/mL should be diluted more.

D. Standard Procedure for the Assay (Fig.1)

Samples should be determined in duplicate.
Make work sheet with Standard Solutions and samples as shown in Fig.2.
A standard curve should be drawn individually for each plate.

1) First incubation:

Add 100 μ L of Standards (10-500 ng/mL), Sample Diluent as 0 ng/mL and diluted samples to the each well.
Incubate the plate at 20 - 25 $^{\circ}$ C for 30 min.

2) Washing:

Remove Standard or sample from the each well.
Add 300 μ L of prepared Washing Solution to the each well.
Remove the Washing Solution from the each well.
Repeat the above steps two times.

3) Second incubation:

Add 100 μ L of Anti-Mouse IgE Enzyme Conjugate to each well.
Incubate the plate at 20 - 25 $^{\circ}$ C for 30 min.

4) Washing:

Follow the same procedure in step 2).

5) Color Development:

Add 100 μ L of Chromogen Substrate to each well.
Incubate the plate at 20 - 25 $^{\circ}$ C for 15 min.
Add 100 μ L of Stop Solution to each well.

6) Absorbance Measurement:

Measure absorbance at 450 nm on each well.

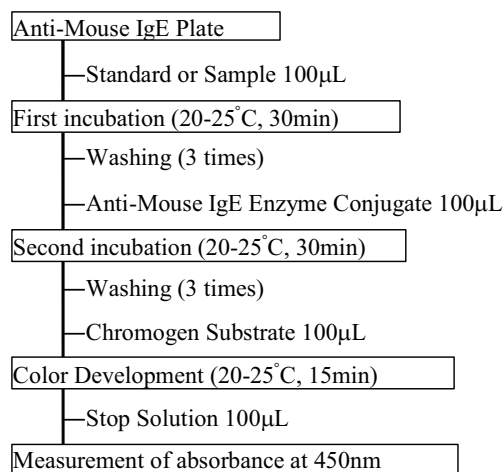


Fig.1 Flow chart of the assay procedure

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 500		Sample#3									
B	Std 100											
C	Std 50											
D	Std 25											
E	Std 10											
F	Std 0											
G	Sample#1											
H	Sample#2											

Fig.2 Example of work sheet

E. Calculation of Results

Plot standard concentrations and absorbance as shown in Fig.3.

Calculate mouse IgE levels in unknown samples by the interpolation from the standard curve.

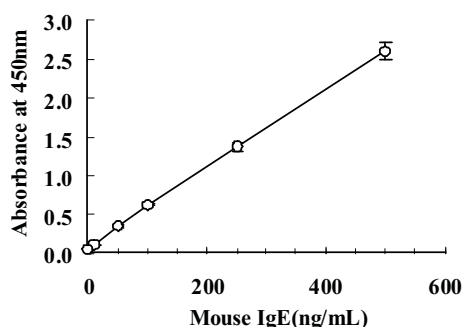


Fig.3 Standard curve of mouse IgE

PRECAUTION FOR USE OR HANDLING OF THE EIA

- 1) The assay procedure should be performed at designated temperature and time.
- 2) The Stopping solution, containing hydrochloride and sulfuric acid, should be handled with care.

STORAGE AND STABILITY

This kit is stable for 18 months at 2 - 8 °C.

REFERENCE

- 1) Hirano, T. et al, Int.Archs Allergy Appl.Immun. 85:47,1988.
- 2) Hirano, T. et al, Med. Immunol. 15:211,1988.

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