



EAGLE
BIOSCIENCES

Mouse IgE ELISA KIT

Catalog Number:

IGE11-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 1.0

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INTENDED USE

The Eagle Biosciences Mouse IgE ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of mouse IgE concentrations in cell culture supernates, serum, and plasma. The Eagle Biosciences Mouse IgE ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

There are five classes of mammalian immunoglobulins: IgA, IgD, IgE, IgG, and IgM. In mice, the IgG class is further divided into four subclasses: IgG1, IgG2a/ IgG2c (strain specific), IgG2b, and IgG3. The general immunoglobulin structure is described as two heavy and two light chain (H₂L₂) polypeptides linked together by disulfide bridges of cysteine residues. Mouse IgE, also known as reaginic antibody, exists in serum as a 4-chain monomer of 185–200 kDa. Like IgM, the IgE monomers contain a fourth constant region domain. Serum concentrations of IgE are reported to be >10 ug/ml. IgE is cytolytic for mast cells, where it binds to the FcRI receptor and is able to mediate the response of granular and lipid mediators of inflammation, including the PCA reaction. The IgE class switch is induced by IL-4 as part of the Th2 response.

PRINCIPLE OF THE ASSAY

The Eagle Biosciences Mouse IgE ELISA Assay Kit employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IgE has been pre-coated onto a microplate. Standard, control, or sample and the working solution of Biotin-Conjugate are pipetted into the wells. Following incubation and wash steps any IgE present is bound by the immobilized antibody and the detection antibody specific for IgE is binds to the combination of capture antibody- IgE in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A colored product is formed in proportion to the amount of IgE present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven IgE standard dilutions and IgE sample concentration determined.

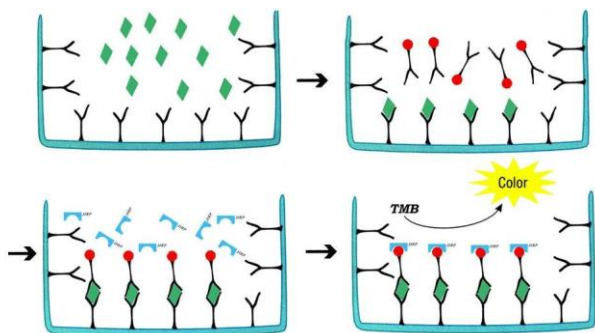


Figure 1: Schematic diagram of the assay

REAGENTS

1. Aluminum pouches with a Microwell Plate coated with antibody to mouse IgE (8x12)
2. 2 vials mouse IgE Standard lyophilized, 20000 pg/ml upon reconstitution
3. 2 vials concentrated Biotin-Conjugate anti-mouse IgE antibody
4. 2 vials Streptavidin-HRP solution,
5. 1 bottle Standard /sample Diluent
6. 1 bottle Biotin-Conjugate antibody Diluent
7. 1 bottle Streptavidin-HRP Diluent
8. 1 bottle Wash Buffer Concentrate 20x (PBS with 1% Tween-20)
9. 1 vial Substrate Solution
10. 1 vial Stop Solution
11. 3 pieces Adhesive Films
12. package insert

NOTE: [96 Tests]

STORAGE

Table 1: Storage of the Mouse IgE ELISA Assay Kit

Unopened Kit	Store at 2 - 8° C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Standard /sample Diluent	May be stored for up to 1 month at 2 - 8° C.**
	Concentrated Biotin-Conjugate	
	Streptavidin-HRP solution	
	Biotin-Conjugate antibody Diluent	
	Streptavidin-HRP Diluent	
	Wash Buffer Concentrate 20x	
	Substrate Solution	
	Stop Solution	
	Standard	Aliquot and store for up to 1 month at ≤20°C. Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.**

**Provided this is within the expiration date of the kit.

THE REQUIRED ITEMS (not provided):

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000ul.
3. 37°C incubator, double-distilled water or deionized water, coordinate paper, graduated cylinder.

PRECAUTIONS FOR USE

1. Store Mouse IgE ELISA Assay Kit reagents between 2°and 8°C. After use all reagents should be immediately returned to cold storage (2°and 8°C).
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this Mouse IgE ELISA Assay Kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with Mouse IgE ELISA Assay Kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.
5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents of the Mouse IgE ELISA Assay Kit and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents of the Mouse IgE ELISA Assay Kit with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.
11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This Mouse IgE ELISA Assay Kit method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

SAMPLE COLLECTION AND STORAGE

1. **Cell Culture Supernates** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.
4. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).
Note: Normal mouse serum or plasma samples are suggested to make a 1:2 dilution.

REAGENT PREPARATION

1. Bring all reagents of the Mouse IgE ELISA Assay Kit to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard** - Reconstitute the Standard with 1mL of Standard /sample Diluent. This reconstitution produces a stock solution of 20000 pg /mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500µL of Standard/sample Diluent into the 10000 pg/mL tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 20000 pg/mL standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0 pg/mL).

If you do not run out of re-melting standard, store it at -20°C. Diluted standard shall not be reused.
4. Working solution of Biotin-Conjugate anti-mouse IGE antibody:
Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

The working solution should be used within one day after dilution.

5. Working solution of Streptavidin-HRP: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

The working solution should be used within one day after dilution.

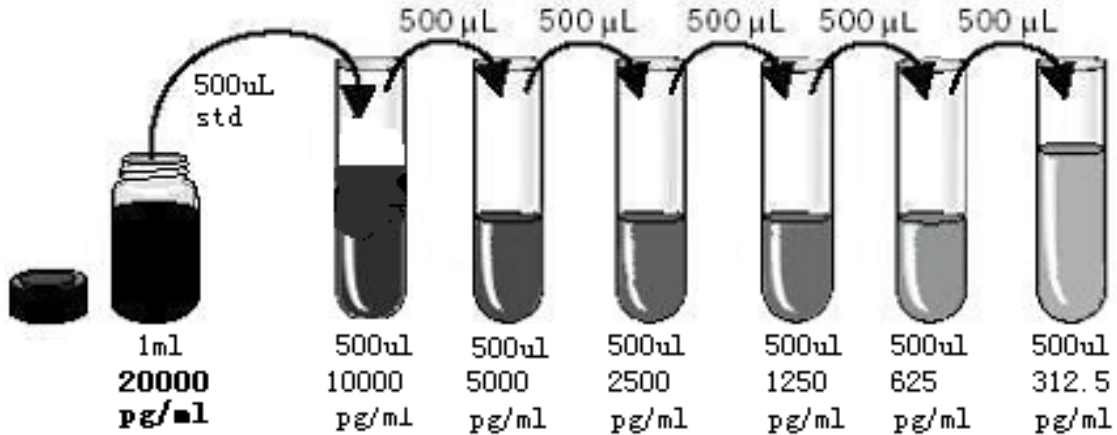


Figure 2: Preparation of IGE standard dilutions

GENERAL ELISA PROTOCOL

1. Prepare all reagents and working standards as directed in the previous sections.
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. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8°C sealed tightly.
3. Add 100 µl of Standard, control, or sample, per well, then add 50 µl of the working solution of Biotin-Conjugate to each well. Cover with the adhesive strip provided and incubate 2 hours at RT. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350 µl) using a squirt bottle, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µl of the working solution of Streptavidin-HRP to each well. Cover with a new adhesive strip and incubate for 30 minutes at RT. Avoid placing the plate in direct light.
6. Repeat the aspiration/wash.
7. Add 100 µl of Substrate Solution to each well. Incubate for 10-20 minutes at RT. Avoid placing the plate in direct light.
8. Add 100 µl of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. (optionally 630nm as the reference wave length; 610-650nm is acceptable)

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.
2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. Create a standard curve by reducing the data using computer software capable of generating 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.
3. The data may be linearized by plotting the log of the IgE concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
4. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Table 2: Typical data using the Mouse IgE ELISA Assay Kit (Measuring wavelength:450nm, Reference wavelength:630nm)

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.114	0.112	0.113	—
312.5	0.152	0.151	0.152	0.156
625	0.199	0.201	0.200	0.197
1250	0.283	0.279	0.281	0.279
2500	0.452	0.449	0.451	0.438
5000	0.728	0.722	0.725	0.743
10000	1.307	1.301	1.304	1.297
20000	2.180	2.189	2.185	2.186

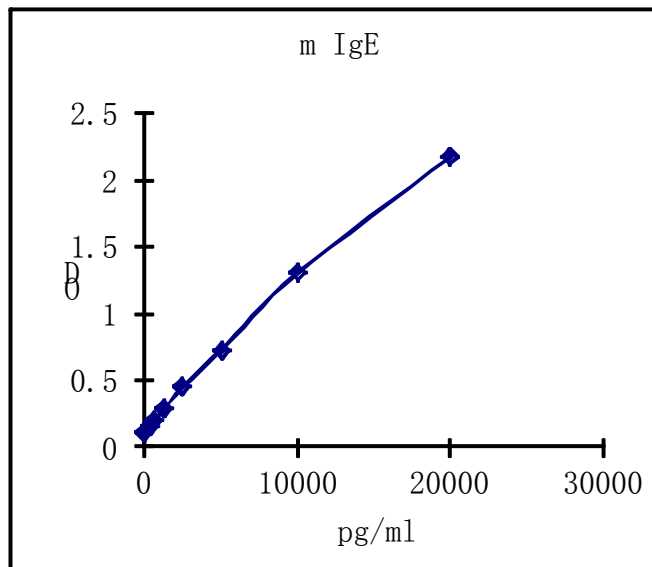


Figure 4: Representative standard curve for Mouse IgE ELISA Assay Kit. IgE was diluted in serial two-fold steps in Sample Diluent.

SENSITIVITY, SPECIFICITY AND REPEATABILITY

1. **REPEATABILITY:** The coefficient of variation of both intra-assay and inter-assay were less than 10%.
2. **SENSITIVITY:** The minimum detectable dose was 156 pg/mL.

REFERENCES

1. Quimby, F. W. & Luong Richard, H. (2007). Clinical Chemistry of the Laboratory Mouse. In J.G.Fox, S. W. Barthold, M. T. Davisson, C. E. Newcomer, F. W. Quimby, & A. L. Smith (Eds.), The Mouse in Biomedical Research: Vol. III Normative Biology, Husbandry, and Models (pp. 190). Burlington: Academic Press.

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