IgE (Mouse) ELISA Kit

Catalog Number KA1944

96 assay

Version: 12

Intended for research use only

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Introduction

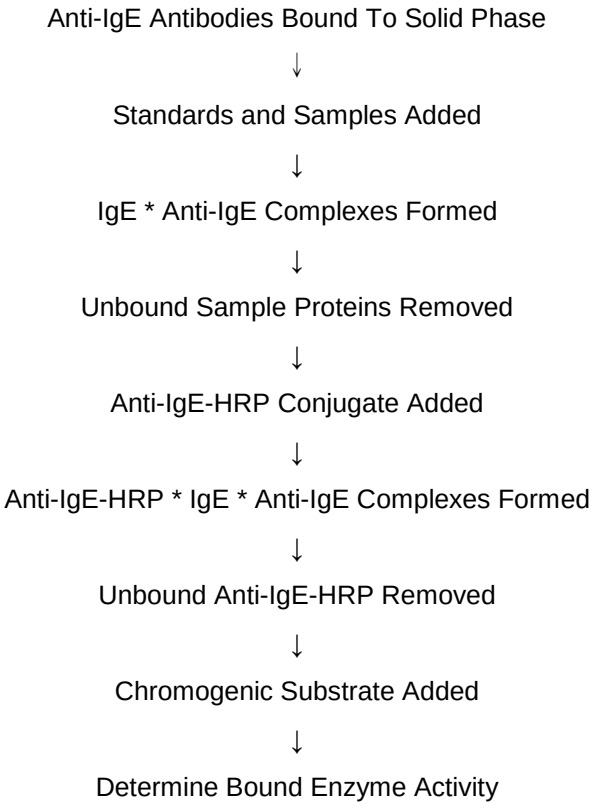
Intended Use

The IgE (Mouse) ELISA Kit is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for measuring IgE in biological samples of mice.

Principle of the Assay

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the IgE present in samples reacts with the anti-IgE antibodies which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, anti-IgE antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the previously bound IgE. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of IgE in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of IgE in the test sample. The quantity of IgE in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

Figure 1.



General Information

Materials Supplied

List of component

Component	Amount
Diluent Concentrate (Running Buffer): One bottle containing 5X concentrated diluent running buffer.	50 mL
Wash Solution Concentrate: One bottle containing a 20X concentrated wash solution	50 mL
Enzyme-Antibody Conjugate 100x: One vial containing affinity purified anti-Mouse IgE antibody conjugated with horseradish peroxidase in a stabilizing buffer	150 µL
Chromogen-Substrate Solution: One vial containing 3,3',5,5'-tetramethybenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.	12 mL
Stop Solution: One vial containing 0.3 M sulfuric acid. WARNING: Avoid contact with skin.	12 mL
Anti-Mouse IgE ELISA Micro Plate: Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with affinity purified anti-Mouse IgE.	96 (8x12) wells
Mouse IgE Calibrator: One vial containing a lyophilized Mouse IgE calibrator	1 vial

Storage Instruction

The expiration date for the package is stated on the box label.

- ✓ Diluent
  The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4-8°C.
- ✓ Wash Solution
  The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25°C) or at 4-8°C.
- ✓ Enzyme-Antibody Conjugate
  Undiluted horseradish peroxidase anti-IgE conjugate should be stored at 4-8°C and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark.
- ✓ Chromogen-Substrate Solution
  The Substrate Solution should be stored at 4-8°C and is stable until the expiration date.
- ✓ Stop Solution
  The Stop Solution should be stored at 4-8°C and is stable until the expiration date.
- ✓ Anti-mouse IgE ELISA Micro Plate
  Anti-Mouse IgE coated wells are stable until the expiration date, and should be stored at 4-8°C in sealed foil pouch with desiccant pack.

✓ Mouse IgE Calibrator

The lyophilized Mouse IgE calibrator should be stored at 4°C or frozen until reconstituted. The reconstituted calibrator should be aliquoted out and stored frozen (avoid multiple freeze-thaw cycles). The working standard solutions should be prepared immediately prior to use.

Materials Required but Not Supplied

- ✓ Precision pipettes (2 µL to 200 µL) for making and dispensing dilutions
- ✓ Test tubes
- ✓ Microplate washer/aspirator
- ✓ Distilled or Deionized H_2O
- ✓ Microtitre plate reader
- ✓ Assorted glassware for the preparation of reagents and buffer solutions
- ✓ Timer

Precautions for Use

✓ Precautions

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

✓ Additives and Preservatives

No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

✓ Known interfering substances

Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

✓ Limitation of the procedure

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.
- Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature.
- Do not mix or substitute reagents with those from other lots or sources.

Assay Protocol

Reagent Preparation

- ✓ Diluent Concentrate

  The Diluent solution supplied is a 5X concentrate and must be diluted 1/5 with distilled or deionized water. (1 part buffer concentrate, 4 parts dH_2O).

- ✓ Wash Solution Concentrate

  The Wash Solution supplied is a 20X concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH_2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

- ✓ Enzyme-Antibody Conjugate

  Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

- ✓ Chromogen-Substrate Solution

  Ready to use as supplied.

- ✓ Stop Solution

  Ready to use as supplied.

- ✓ Anti-Mouse IgE ELISA Micro Plate

  Ready to use as supplied. Unseal Microtiter Pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

- ✓ Mouse IgE Calibrator

  Add 1.0 mL of distilled or de-ionized water to the Mouse IgE Calibrator and mix gently until dissolved. The calibrator is now at a concentration of 1.875 µg/mL (the reconstituted calibrator should be aliquoted and frozen if future use is intended). Mouse IgE standards need to be prepared immediately prior to use (See chart below). Mix well between each step. Avoid foaming.

Standard	ng/mL	Volume added to 1X Diluent	Volume of 1X Diluent
7	400	160 µL Mouse IgE Calibrator	590 µL
6	200	300 µL Calibrator 7	300 µL
5	100	300 µL Calibrator 6	300 µL
4	50	300 µL Calibrator 5	300 µL
3	25	300 µL Calibrator 4	300 µL
2	12.5	300 µL Calibrator 3	300 µL
1	6.25	300 µL Calibrator 2	300 µL
0	0		600 µL

Sample Preparation

✓ Specimen Collection and Handling

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freezing-thaw cycles.

✓ Dilution of Samples

The assay for quantification of IgE in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1/50 is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

To prepare a 1/50 dilution of sample, transfer 5 µL of serum sample to 245 µL of 1X Diluent. This gives you a 1/50 dilution. Mix thoroughly.

Assay Procedure

1. Bring all reagents to room temperature before use.
2. Pipette 100 µL of
   - Standard 0 (0.0 ng/mL) in duplicate
   - Standard 1 (6.25 ng/mL) in duplicate
   - Standard 2 (12.5 ng/mL) in duplicate
   - Standard 3 (25 ng/mL) in duplicate
   - Standard 4 (50 ng/mL) in duplicate
   - Standard 5 (100 ng/mL) in duplicate
   - Standard 6 (200 ng/mL) in duplicate
   - Standard 7 (400 ng/mL) in duplicate
3. Pipette 100 µL of sample (in duplicate) into predesignated wells.
4. Incubate the microtiter plate at room temperature for thirty (30 ± 2) minutes. Keep plate covered and level during incubation.
5. Following incubation, aspirate the contents of the wells.
6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for thirty (30 ± 2) minutes. Keep plate covered in the dark and level during incubation.
8. Wash and blot the wells as described in Steps 5/6.
9. Pipette 100 µL of TMB Substrate Solution into each well.
10. Incubate in the dark at room temperature for precisely ten (10) minutes.
11. After ten minutes, add 100 µL of Stop Solution to each well.
12. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to manufacture's specifications.

✓ Stability of the final reaction mixture

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

Data Analysis

Calculation of Results

1. Subtract the average background value from the test values for each sample.
2. Using the results observed for the standards construct a Standard Curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.
3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the IgE concentration in original samples.

Performance characteristics

- ✓ Indications of instability

  If the test is performing correctly, the results observed with the standard solutions should be within 20% of the expected values.

Plate Layout

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7								
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1								
	A	B	C	D	E	F	G	H