Rat IgE ELISA
Life Diagnostics, Inc., Catalog Number: IGE-2

INTRODUCTION

IgE is the least abundant immunoglobulin in mammalian serum. In rats, basal IgE levels vary with strain, ranging from approximately 0.1 to 3 μg/ml, and can increase 20-100-fold in response to allergens or infection.[1,2] Maximal responses are observed approximately 25-40 days after immunologic stimulus.

PRINCIPLE OF THE ASSAY

The rat IgE ELISA kit is designed for the measurement of IgE in serum and plasma. The assay uses goat anti-rat IgE for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated goat anti-rat IgE antibodies for detection.

Test samples are diluted and incubated in the microtiter wells for 45 minutes alongside prepared rat IgE standards. The microtiter wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. IgE molecules are thus sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies. TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow. Optical density is measured spectrophotometrically at 450 nm. The concentration of IgE is proportional to the optical density of the test sample and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-Rat IgE Coated 96-well Plate (12 strips of 8 wells)
- HRP Conjugate Reagent, 11 ml
- Reference Standard (lyophilized)
- 20x Wash Solution: TBS50-20, 50 ml
- 10x Immunoglobulin Diluent: RD25-10, 25 ml
- TMB Reagent (One-Step): TMB11-1, 11 ml
- Stop Solution (1N HCl): SS11-1, 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-plate incubator/shaker mixing speed of 150 rpm
- Plate washer
- Plate reader with an optical density range of 0-4 at 450nm
- Graph paper (PC graphing software is optional)

STORAGE

The lyophilized reference standard should be stored at or below -20°C for optimum stability (it can be safely shipped at 4°C). The remainder of the kit should be stored at 4°C. The test kit will remain stable for six months from the date of purchase provided that the components are stored as described above.

The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (25°C) before use.
3. Optimum results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use, estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use, dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The IgE reference standard is provided as a lyophilized stock. Reconstitute as instructed on the standard vial. ***(The reconstituted standard should be aliquoted and frozen at -20°C after reconstitution if future use is intended)***.
2. Label 8 polypropylene or glass tubes as 100, 50, 25, 12.5, 6.25, 3.13, 1.56, and 0 ng/ml.
3. Into the tube labeled 100 ng/ml, pipette the volume of diluent detailed on the IgE standard vial label. Then add the indicated volume of IgE standard (shown on the IgE standard vial label) and mix gently. This provides the 100 ng/ml standard.
4. Dispense 250 μl of diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.13, 1.56, and 0 ng/ml.
5. Prepare a 50 ng/ml standard by diluting and mixing 250 μl of the 100 ng/ml standard with 250 μl of diluent in the tube labeled 50 ng/ml.
6. Similarly prepare the 25, 12.5, 6.25, 3.13, and 1.56 ng/ml standards by serial dilution.

SAMPLE PREPARATION

Because serum levels of IgE vary with rat strain and immunologic stimulus, optimal dilution of samples must be determined empirically. However, samples must be diluted at least 40-fold to eliminate matrix effects.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μl of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 45 minutes.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400 μl/well). The entire wash procedure should be performed as quickly as possible.

5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
6. Add 100 µl of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 45 minutes.
8. Wash as detailed in 4 to 5 above.
9. Dispense 100 µl of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
13. Read the optical density at 450 nm with a microtiter plate reader *within 5 minutes*.

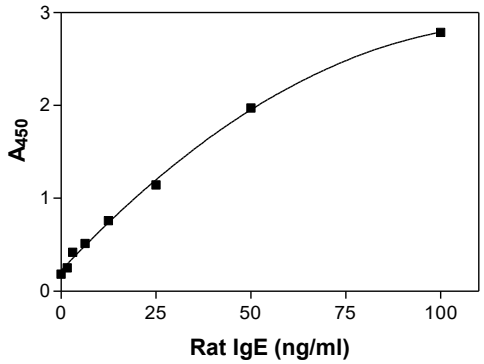
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of IgE in ng/ml from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgE in the sample.
5. PC graphing software may be used for the above steps.
6. If the OD_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y-axis against IgE concentrations on the X-axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

IgE (ng/ml)	A_{450}
100	2.787
50	1.971
25	1.144
12.5	0.758
6.25	0.515
3.13	0.418
1.56	0.251
0	0.183



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions detailed above.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

REFERENCES

1. Rousseaux-Prevost R, Bazin H, and Capron A. IgE in experimental schistosomiasis: 1. Serum IgE levels after infection by *Schistosoma Mansoni* in various strains of rats. Immunology 33:501-505 (1977)
2. Thorpe SC, Murdoch MD, and Kemeny DM. The effect of castor bean toxin, ricin on rat IgE and IgG responses. Immunology 68:307-311 (1989)

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For technical assistance please email us at
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