Monkey IgE ELISA
Life Diagnostics, Inc., Catalog Number: IGE-3

FOR RESEARCH USE ONLY

INTRODUCTION

The monkey IgE ELISA kit is designed for measurement of IgE in old world monkey serum or plasma. The assay uses a mouse monoclonal anti-monkey IgE antibody for solid phase (microtiter wells) immobilization and a different horseradish peroxidase (HRP) conjugated mouse monoclonal anti-monkey IgE antibody for detection. The kit will also recognize human IgE, but this kit is not intended for research or diagnostic testing of human samples. Cross-reactivity with IgE from other species has not been investigated.

IgE is the least abundant immunoglobulin in serum, typically present at levels of 1 μg/ml per ml or lower. It is involved in allergic reactions, binding to Fc receptors on basophils and mast cells. Subsequent binding of antigen to IgE triggers release of histamine and other vasoactive amines. IgE levels are elevated in asthma, eczema, rhinitis, and parasitic infections. IgE is useful as a serum biomarker in such conditions.

PRINCIPLE OF THE ASSAY

Test samples are diluted and incubated in the microtiter wells for 45 minutes alongside prepared monkey IgE standards. The microtiter wells are subsequently washed, and 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, and 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 monkey IgE coated 96-well plate (12 strips of 8 wells)
- HRP Conjugate Reagent, 11 ml
- Reference standard (lyophilized)[1] **Store at -20°C**
- 20x Wash Solution: TBS50-20, 50 ml
- 10x 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 in a freezer at or below -20°C when the kit is received. **The remainder of the kit should be stored in a refrigerator at 4°C and must not be frozen**. 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 with 1.0 ml of distilled or deionized water ***(the reconstituted standard is stable at 4°C for one day but should be aliquoted and frozen at -20°C after reconstitution if future use is intended)***.
2. Label 5 polypropylene or glass tubes as 40, 20, 10, 5, and 2.5 ng/ml.
3. Into the tube labeled 40 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 40 ng/ml standard.
4. Dispense 250 μl of diluent into the tubes labeled 40, 20, 10, 5, and 2.5 ng/ml.
5. Prepare a 20 ng/ml standard by diluting and mixing 250 μl of the 40 ng/ml standard with 250 μl of diluent in the tube labeled 20 ng/ml.
6. Similarly prepare the 10, 5, and 2.5 ng/ml standards by serial dilution.

[1] The IgE standard used in this kit is of non-monkey origin. It behaves identically to old-world monkey IgE. The use of a non-monkey standard allows export of the kit without requirement for CITES documentation.

SAMPLE PREPARATION

General Note: We found IgE to present in a panel of Rhesus and Cynomolgus monkey serum samples at concentrations of approximately 0.2 to 10 μg/ml, with most samples in the 0.2 to 1 μg/ml range. To obtain values within range of the standard curve, we suggest that samples initially be diluted 200-fold using the following procedure for each sample to be tested:

1. Dispense 248.75 μl of diluent into a microcentrifuge tube.
2. Pipette and mix 1.25 μl of the serum/plasma sample into the 248.75 μl of diluent. This provides a 200-fold diluted sample.

Do not use sample dilutions less than 40-fold (i.e., 20-fold). At dilutions less than 40-fold, serum factors may interfere with the assay.

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*.

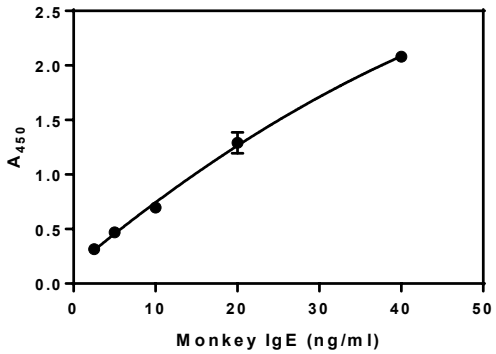
CALCULATION OF RESULTS

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. Please note that we perform validation of all assays using shaking incubators at 150 rpm and 25°C. Performance of the assay at lower mixing speeds and temperatures may result in lower absorbance values.

IgE (ng/ml)	A_{450}
40	2..080
20	1.291
10	0.696
5	0.470
2.5	0.315



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.

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For technical assistance please email us at
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