Human IgE ELISA
Life Diagnostics, Inc., Catalog Number: IGE-20

USE STATEMENT

The human IgE ELISA kit is for research use only. Under no circumstances may it be used for diagnostic purposes.

INTRODUCTION

The human IgE ELISA kit is for measurement of IgE in human serum[1] and fluids. The assay uses two monoclonal antibodies developed at Life Diagnostics; one for solid phase immobilization (microtiter wells), the other, conjugated to HRP, is used for detection.

IgE is the least abundant immunoglobulin in serum, typically present at levels of approximately 100 ng/ml. 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.

PRINCIPLE OF THE ASSAY

Diluted serum samples and standards are incubated in the microtiter wells for one hour with HRP conjugate. If IgE molecules are present, they are sandwiched between the immobilization and detection antibodies. The wells are then washed. TMB reagent is added and incubated for 20 minutes. This results in the development of a blue color if IgE is present. Color development is stopped by the addition of Stop Solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentrations of IgE in samples are proportional to absorbance and are derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-human IgE coated 96-well plate (12 x 8 well strips)
- HRP Conjugate, 11 ml
- IgE Stock (lyophilized) **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
- Plate incubator/shaker
- Plate washer
- Plate reader with an optical density range of 0-4 at 450 nm
- Graphing software

STORAGE

The lyophilized IgE stock should be stored in a freezer at -20°C when the kit is received. The rest of the kit should be stored in a refrigerator at 4°C. The kit will remain stable for at least six months from the date of purchase provided that the components are stored as described. 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. Estimate the 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. Occasionally, crystals may form in the stock. If present, dissolve them by warming the stock in lukewarm water prior to use.

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. Reconstitute the IgE stock with 1.0 ml of distilled or deionized water (the reconstituted stock is stable at 4°C for one day but should be aliquoted and frozen at -20°C if future use is intended).
2. Label six microcentrifuge tubes as 200, 100, 50, 25, 12.5 and 6.25 ng/ml.
3. Into the tube labeled 200 ng/ml, pipette the volume of diluent detailed on the IgE stock vial label. Then add the indicated volume of IgE stock and mix gently. This gives the 200 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 100, 50, 25, 12.5 and 6.25 ng/ml.
5. Prepare the 100 ng/ml standard by diluting and mixing 250 µl of the 200 ng/ml standard with 250 µl of diluent in the tube labeled 100 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

We found IgE concentrations ranging from less than 50 ng/ml to 15,000 ng/ml in serum. Optimal dilutions should be determined empirically, but we suggest testing samples initially at a 40-fold dilution. To avoid matrix effects, do not use sample dilutions less than 20-fold (i.e., 10-fold).

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of HRP conjugate into the wells.
3. Dispense 100 µl of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
4. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for one hour.
5. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400

[1] Plasma has not yet been evaluated in the assay but will likely be compatible if tested at dilutions of 50-fold or greater.

μl/well). The entire wash procedure should be performed as quickly as possible.
6. If necessary, strike the wells sharply onto absorbent paper or paper towels to remove residual wash buffer.
7. Dispense 100 μl of TMB Reagent into each well.
8. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 20 minutes.
9. Stop the reaction by adding 100 μl of Stop Solution to each well.
10. Gently mix. It is important to make sure that all the blue color changes to yellow.
11. Measure the absorbance at 450 nm with a microtiter plate reader within 5 minutes.

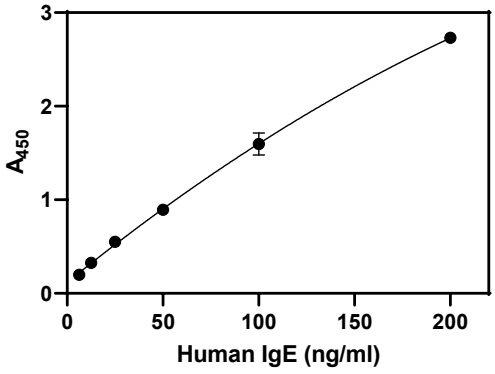
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus concentration.
2. Fit the standard curve to and appropriate model and determine the concentration of the samples from the standard curve. We use a second order polynomial (quadratic) fit.
3. Multiply the derived concentration by the dilution factor to determine the concentration in the serum sample.
4. If the A_{450} values of samples fall outside or at the extremes of the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with absorbance at 450nm on the Y-axis and 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 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}
200	2.730
100	1.595
50	0.895
25	0.551
12.5	0.325
6.25	0.199

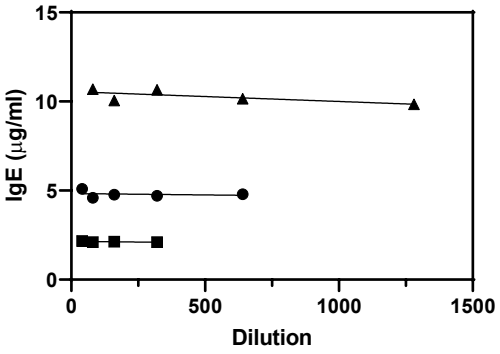


LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is performed 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.

ASSAY PERFORMANCE

Parallelism: To assess performance of the assay, three human ascites samples with IgE concentrations of 10.28, 4.8 and 2.13 μg/ml were serially diluted to produce values within the dynamic range of the assay.



Cross reactivity: The assay is specific for IgE. Human IgG was non-reactive when tested at 100 μg/ml. When tested at concentrations of 10 μg/ml, human IgA and IgM were non-reactive. Higher concentrations were not tested.

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For technical assistance please email us at
techsupport@lifediagnostics.com