Rat IgE ELISA Kit

Enzyme Immunoassay for the quantification of Rat Immunoglobulin E in serum, plasma, cell culture supernatants

Catalog number: ARG80248

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Immunoglobulin E (IgE) is a 180 kDa soluble protein serving as an antigen-specific unit of mast cell effector mechanisms. IgE has the lowest serum concentration of all immunoglobulins (approximately 0.5 mg/l) in healthy individuals, but upon allergen challenge its concentration in blood increases dramatically. Although biological survival of free IgE is very short (T1/2 = 2 days), it is stabilized after binding to its high affinity receptor. Unlike IgM- IgG- and IgA-committed B cells, IgE-switched B cells do not undergo clonal expansion.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for IgE has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any IgE present is bound by the immobilized antibody. After washing away any unbound substances, a HRP-conjugated antibody specific for IgE is added to each well and incubate. Following a washing to remove unbound substances, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of IgE bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of IgE in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Lyophilized)	3 X 250 ng/vial	4°C
Standard diluent buffer	20 ml	4°C
Antibody conjugate concentrate	1 vial (400 µl)	4°C
Antibody diluent buffer	16 ml	4°C
20X Wash buffer	50 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
STOP solution	12 ml	4°C
Plate sealer	6 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.

- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

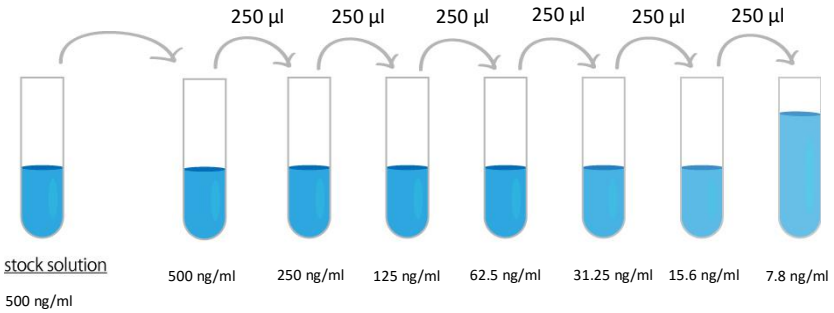
Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.
- 1X Antibody conjugate: Dilute 30X antibody conjugate concentrate into 1X antibody diluent buffer to yield 1X Detection antibody solution.
- Standards: Reconstitute the standard with 500 µl standard diluent buffer

to yield a stock concentration of 500 ng/ml. Make sure the standard is dissolved completely before making serial dilutions. The standard diluent buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 500 ng/ml, 250 ng/ml, 125 ng/ml, 62.5 ng/ml, 31.25 ng/ml, 15.6 ng/ml, 7.8 ng/ml.



ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards, samples and zero controls (standard diluent buffer) into wells. Incubate for 1.5 h at 36 °C.
3. Aspirate each well and wash, repeating the process four times for a total five washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash,

remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

4. Add 100 µl 1X Antibody conjugate into each well. Cover wells and incubate for 1 hour at 36°C.
5. Aspirate each well and wash as step 3.
6. Add 100 µl of TMB Reagent to each well. Incubate for 15 minutes at 36°C in dark.
7. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

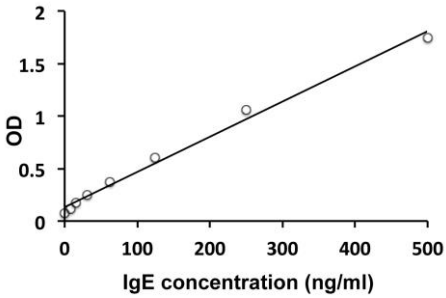
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Rat IgE ranged from 7.8-500 ng/ml. The mean MDD was 4 ng/ml.

Specificity

This assay recognizes natural and recombinant Rat IgE. No significant cross-reactivity or interference with the factors below was observed:

Rat IL-1,2,3,5,6,8,10, IFN, TNF ; mouse IgE, human IgE

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.