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Human IgE ELISA Kit

Strip well format. Reagents for up to 96 tests

Catalog No.	**CS221A**	**Quantity:**	**1 x 96 tests**
	CS221B		**5 x 96 tests**

Intended Use: This human immunoglobulin E antigen assay is intended for the quantitative determination of total human IgE antigen in serum, plasma, hybridoma cell supernatants, ascites or other biological fluids.

Background: IgE is the least abundant immunoglobulin in serum and is predominately involved in the allergy response. IgE binds to allergens and triggers histamine release from mast cells and basophils. Elevated IgE levels are found in patients experiencing severe allergic reactions and parasitic infections.

Assay Principle: Human IgE will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, horseradish peroxidase labeled polyclonal anti-human IgE antibody binds to the captured protein. Excess antibody is washed away and TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human IgE. Color development is proportional to the concentration of IgE in the samples.

Definition of International Unit: Total serum IgE concentrations are reported in international units or nanograms per milliliter. Conversion Factor: 1 IU/ml = 3.4 ng/ml as experimentally calibrated against the WHO Reference Reagent for Human Serum Immunoglobulin E (IgE), lot 75/502 distributed by NIBSC, Hertfordshire, United Kingdom.

Reagents Provided:

♦ Antibody Coated Plate:
1-96 well immulon strip plate (8X12 removable strips) coated with IgE capture antibody, blocked, and dried
♦10X Wash Buffer:
1 bottle of 50ml; bring to 1X using DI water
♦ Human IgE antigen standard:
1 vial of lyophilized standard
♦ Peroxidase anti-human IgE antibody:
1 vial of lyophilized HRP labeled antibody
♦¨TMB substrate solution:
1 bottle of 10ml solution

Storage and Stability: All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -70°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

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Reagents and Equipment Required:

- 1-channel pipettes covering 0-10 µl and 200-1000 µl
- 12-channel pipette covering 30-300µl
- Paper towels or kimwipes
- 50 ml tubes, 1.5 ml centrifuge tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm.

Warnings:

Warning – Avoid skin and eye contact when using TMB One substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

Precautions:

- **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- **DO NOT** pipette reagents by mouth.
- Always pour TMB substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the TMB substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

Preparation of Reagents:

- Wash buffer concentrate: The wash buffer is supplied in a 10X concentrate and must be diluted 1:10 with deionized water for use with the kit.
- TBS: 0.1M Tris-HCl, 0.15M NaCl, pH 7.4
- Blocking buffer (BSA): 3% BSA in TBS

Specimen Collection:

The assay measures total human IgE in the 1-500 ng/ml range. Samples giving human IgE levels above 500 ng/ml should be diluted in BSA blocking buffer before use. A 1:10 dilution for plasma is suggested for best results.

Assay Procedure:

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard:

Reconstitute standard vial with 1 ml of BSA blocking buffer to give a 500 ng/ml (147 IU/ml) solution.

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Dilution table for preparation of human IgE standards:

IgE concentration (ng/ml)	Dilutions
500	100µl from standard vial
200	600µl (BSA) + 400µl (500ng/ml)
100	500µl (BSA) + 500µl (200ng/ml)
50	500µl (BSA) + 500µl (100ng/ml)
20	600µl (BSA) + 400µl (50ng/ml)
10	500µl (BSA) + 500µl (20ng/ml)
5	500µl (BSA) + 500µl (10ng/ml)
2	600µl (BSA) + 400µl (5ng/ml)
1	500µl (BSA) + 500µl (2ng/ml)
0.5	500µl (BSA) + 500µl (1ng/ml)
0	500µl (BSA) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition:
Remove microtiter plate from bag. Add 100 µL standards in duplicate and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µL wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

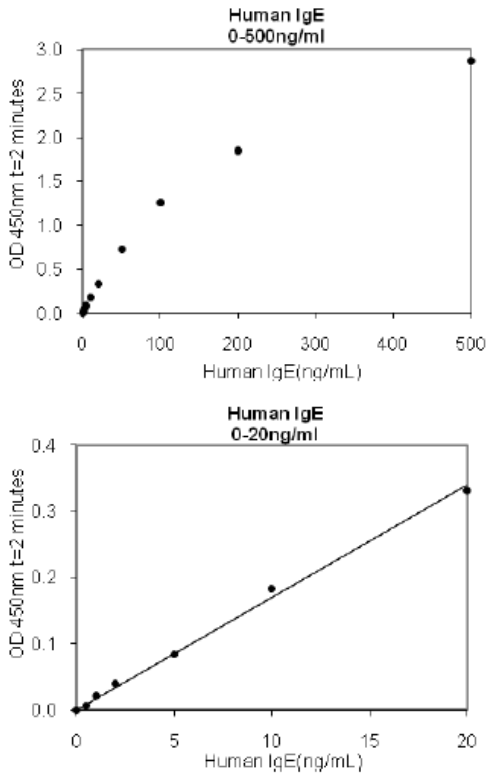
Peroxidase Antibody Addition:
Reconstitute peroxidase conjugated antibody by adding 10ml BSA blocking buffer to vial. Agitate gently to completely dissolve contents. Add 100 µl to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation:
Add 100 µl of TMB substrate solution to all wells and shake plate at 300 rpm for 2-10 minutes. Quench the reaction with the addition of 50 µl of 1N H_2SO_4 and read final absorbance values at 450nm.
NOTE: Time for substrate development is dependent on needs of researcher.

Measurement:
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Assay Calibration:
Plot A_{450} against the amount of human Factor VII in the standards. Fit a straight line through the points using a linear fit procedure. The amount of total human IgE in the unknowns can be determined from this curve..

A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)



Expected Values: The level of IgE in normal human serum is low relative to IgG. Concentrations of 52 ng/ml in single donor plasma and 170 ng/ml in pooled plasma were found by in house testing using a 1:10 dilution. IgE is elevated in allergic and parasitic disease states as well as certain inflammatory and infectious disease states and immunologic disorders.

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Disclaimer: This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling or from contact with the above product.

Example of Kit Plate Layout
96 Well Plate
Standards: 22 wells
Samples: 74 wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml	20ng/ml	50ng/ml	100ng/ml	200ng/ml	500ng/ml	
B	0	0.5ng/ml	1ng/ml	2ng/ml	5ng/ml	10ng/ml	20ng/ml	50ng/ml	100ng/ml	200ng/ml	500ng/ml	
C												
D												
E												
F												
G												
H												

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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