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Human IgE ELISA Kit

Catalog No: CS221A
CS221B

Size: 1 x 96 tests
5 x 96 tests

Sensitivity:	0.38 ng/ml
Specificity:	Total Human IgE
Range:	0.5-500 ng/ml
Sample Type:	Serum, plasma, hybridoma cell supernatants, ascites or other biological fluids
Cross-Reactivity:	Pooled normal plasma from mouse, rat, dog, horse and sheep were assayed and no significant cross-reactivity was observed.

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 450 nm. 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.

Standard Calibration: **Human IgE Standard provided is calibrated against the WHO International Standard for IgE in Human Serum distributed by NIBSC (75/502), South Mimms Potters Bar, Hertfordshire, UK. Lot 315L: 500 ng = 480 IU**

Reagents Provided:

Description	Quantity
CS221A – P. 96-well microtiter strip plate coated with anti- Human IgE antibody, blocked and dried on well surface	1 plate: 96 wells (12 strips x 8 wells)
CS221A - A. Wash Buffer Concentrate (10x)	1 bottle, 50 mL
CS221A - B. Human IgE standard, lyophilized	1 vial
CS221A - C. Anti-Human IgE horseradish peroxidase antibody, lyophilized polyclonal	1 vial
CS221A - D. TMB substrate solution	1 bottle, 10 ml

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Storage and Stability:

All kit components must be stored at 2-8°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -80°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 2-8°C. Kit should be used no later than the expiration date.

Reagents and Equipment Required:

- Pipettes covering 0-10 µl and 200-1000 µl, and tips
- 12-channel pipette covering 30-300µl
- Paper towels or laboratory wipes
- Polypropylene conical 50 ml tubes, 1.5 ml flip-cap tubes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)
- Deionized or distilled water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450 nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm
- Automatic plate washer or wash bottle

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 substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the 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:

- **TBS:** 0.1 M Tris 0.15 M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer concentrate:** Dilute 50 ml of 10X wash buffer with 450 ml deionized water

Specimen Collection:

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or aliquot and store at $\leq$ - 20°C. Avoid repeated freeze-thaw cycles.

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Assay Procedure: Allow microtiter strips and assay components to warm to room temperature for 30 minutes. Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard:
Reconstitute Standard by adding **1.0 ml of blocking buffer** directly to the vial and agitate gently to completely dissolve contents. This will result in a 500 ng/ml standard solution.

Table 1: Dilution table for preparation of Human IgE standard:

IgE Concentration (ng/ml)	Dilutions
500	From Standard vial
200	600 µl (BB) + 400 µl (500ng/ml)
100	500 µl (BB) +500 µl (200 ng/ml)
50	500 µl (BB) + 500 µl (100 ng/ml)
20	600 µl (BB) + 400 µl (50 ng/ml)
10	500 µl (BB) + 500 µl (20 ng/ml)
5	500 µl (BB) + 500 µl (10 ng/ml)
2	600 µl (BB) + 400 µl (5 ng/ml)
1	500 µl (BB) + 500 µl (2 ng/ml)
0.5	500 µl (BB) + 500 µl (1 ng/ml)
0	500 µl (BB) Zero point to determine background

NOTE: Dilutions for the standard curve must be made and applied to the plate immediately.

Standard and Unknown Addition:
Remove microtiter plate from bag. Add 100 µl of IgE 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 laboratory wipes.

NOTE: The assay measures total Human IgE antigen in the 0.5 - 500 ng/ml range. If the unknown is thought to have higher IgE levels, dilutions may be made in **blocking buffer**. A 1:10 dilution for normal Human serum or plasma is suggested for best results.

Primary Antibody Addition:
Briefly centrifuge vial before opening. Dilute **2 µl** of conjugated antibody in **10 ml blocking buffer** and add 100 µl to all wells. 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 laboratory wipe.

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Substrate Incubation:

Add 100 µl TMB substrate to all wells and shake plate for 2-5 minutes. Substrate will change from colorless to different intensities of blue. Quench reaction by adding 50 µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate and read plate immediately.

Measurement:

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

Calculation of Results:

Plot A_{450} against the amount of Human IgE in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four-parameter logistic (4PL) curve fit. The amount of IgE in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

Example of ELISA Plate Layout
96 Well Plate: 22 Standard wells, 74 Sample wells

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

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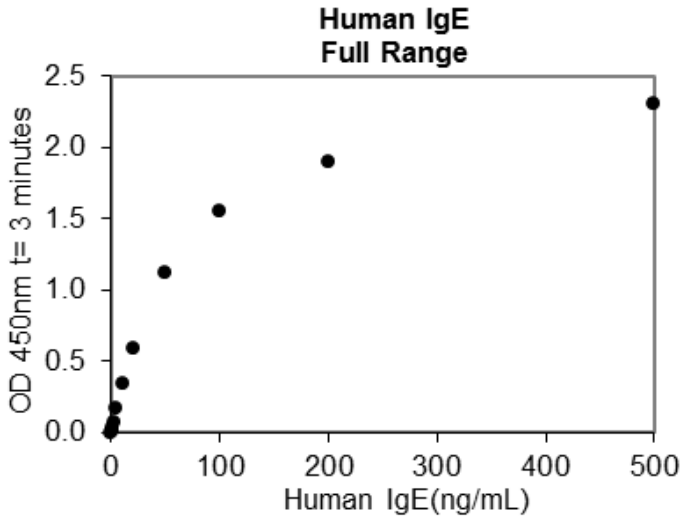
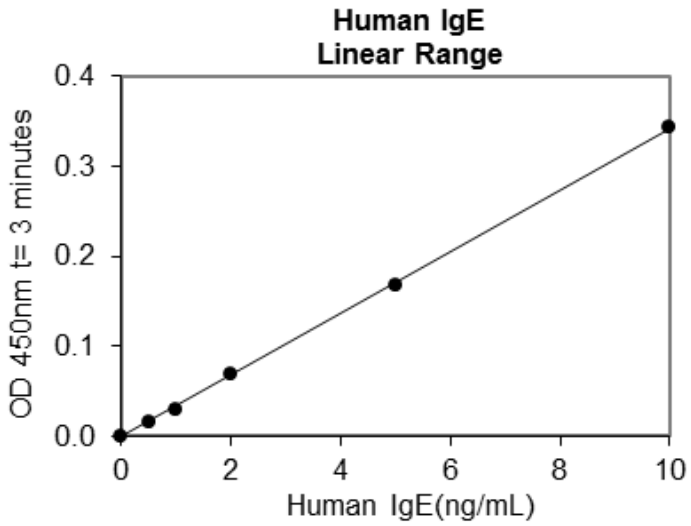
A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





Expected Value: 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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Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.044-0.062) and calculating the corresponding concentration. The MDD was 0.38 ng/ml.

Specificity: This assay recognizes total Human IgE. Pooled normal plasma from mouse, rat, dog, horse and sheep was assayed and no significant cross-reactivity was observed.

Intra-assay Precision: 3 samples of known concentration were tested 20 times on 1 plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	2.5	10.1	50.01
Standard Deviation	0.175	0.632	1.89
CV (%)	6.99	6.26	3.78

Inter-assay Precision: These studies are currently in progress. Please contact us for more information.

Recovery: These studies are currently in progress. Please contact us for more information.

Linearity: These studies are currently in progress. Please contact us for more information.

Disclaimer: This information is believed to be correct but does not claim to be all-inclusive and should 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.

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

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