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Human IgA ELISA Kit

Catalog No: CS163A
CS163B

Size: 1 x 96 tests
5 x 96 tests

Sensitivity:	0.035 ng/ml
Specificity:	Total Human IgA
Range:	0.1-100 ng/ml
Sample Type:	Serum, plasma, hybridoma cell supernatants, ascites or other biological fluids

Background: IgA is the most abundant immunoglobulin in body fluids and the second most abundant immunoglobulin in plasma. IgA in serum is a primarily monomeric 160kDa glycoprotein that initiates defenses against natural infection through interaction with specific receptors and immune mediators. Each monomer consists of two heavy chains and two kappa or lambda light chains. Most serum IgA molecules are subclass IgA1 which have longer hinge regions than subclass IgA2

Assay Principle: Human IgA will bind to the affinity purified capture antibody coated on the microtiter plate. After appropriate washing steps, horseradish peroxidase labeled polyclonal anti-human IgA 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 IgA. Color development is proportional to the concentration of IgA in the samples.

Standard Calibration: Human IgA Standard provided is calibrated against the WHO International Standard for IgA, IgG and IgM in Human Serum distributed by NIBSC (67/086), South Mimms Potters Bar, Hertfordshire, UK. Lot 617L: 500 ng = 0.040 IU

Reagents Provided:

Description	Quantity
CS163A – P. 96-well microtiter strip plate coated with anti- Human IgA antibody, blocked and dried on well surface	1 plate: 96 wells (12 strips x 8 wells)
CS163A - A. Wash Buffer Concentrate (10x)	1 bottle, 50 mL
CS163A - B. Human IgA standard, lyophilized	1 vial
CS163A - C. Anti-Human IgA horseradish peroxidase antibody, lyophilized polyclonal	1 vial
CS163A - 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 IgM standard:

IgA Concentration (ng/ml)	Dilutions
100	800 µl (BB) + 200 µl (from vial)
50	500 µl (BB) + 500 µl (100ng/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)
0.2	600 µl (BB) + 400 µl (0.5 ng/ml)
0.1	500 µl (BB) + 500 µl (0.2 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 IgA 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 IgA antigen in the 0.1 - 100 ng/ml range. If the unknown is thought to have higher IgA levels, dilutions may be made in blocking buffer. A 1:100,000 to 1:800,000 dilution for normal Human serum or plasma is suggested for best results.

Primary Antibody Addition:
Briefly centrifuge vial before opening. Dilute 2.5 µl HRP conjugated antibody into **2.5 ml blocking buffer** to make a 1:1,000 dilution. Add 1 ml of 1:1,000 dilution to 9 ml of blocking buffer to generate at 1:10,000 dilution. 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 2-10 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 IgA 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 IgA 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	0.1 ng/ml	0.2 ng/ml	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	
B	0	0.1 ng/ml	0.2 ng/ml	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	
C												
D												
E												
F												
G												
H												

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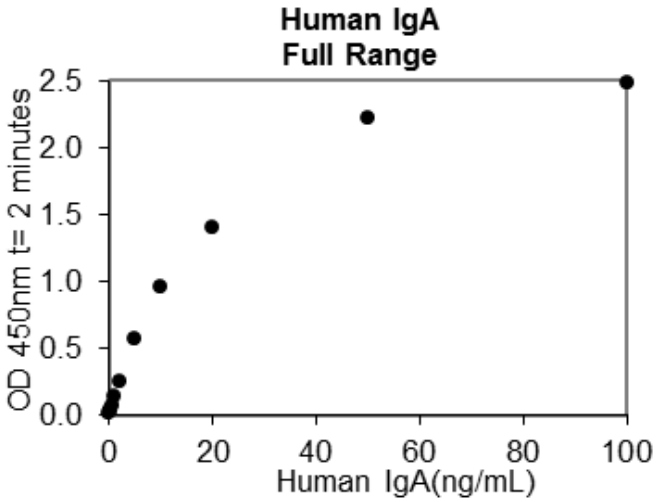
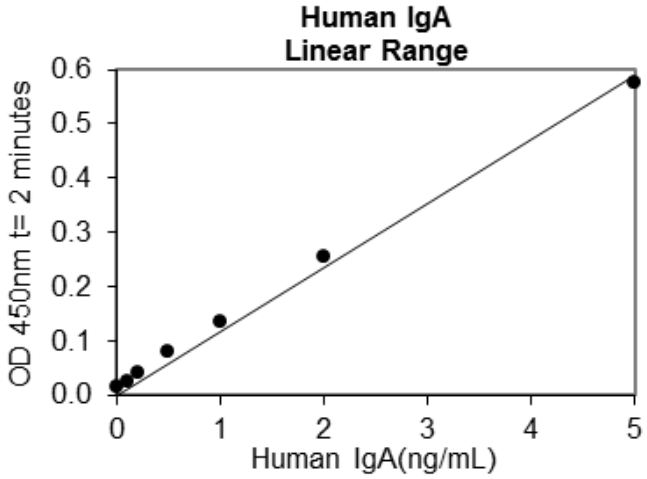
A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





Expected Value:

The concentration of IgA in normal Human serum ranges from 0.7 - 4.0 mg/ml.

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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}: 0.05-0.058) and calculating the corresponding concentration. The MDD was 0.035 ng/ml.

Specificity: This assay recognizes total Human IgA. Cross-reactivity studies with other species are in progress.

Linearity: To assess the linearity of the assay, pooled citrated human plasma containing high concentrations of IgA were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of Expected	96	100	101	111
Range	94 - 99%	98 – 102%	98 – 103%	108 – 114%

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.

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

Sample Values: Samples were evaluated for the presence of IgA at varying dilutions.

Sample Type	Dilution	Mean (ng/ml)
Citrate Plasma	1:200,000	2.061
	1:400,000	2.089
	1:800,000	2.015

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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