Human IgA ELISA Kit

Item No. 502520

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
400561	Anti-Human IgA ELISA HRP Conjugate	1 ea/500 µl	4°C
400563	Anti-Human IgA ELISA Strip Plate	1 plate	4°C
400562	Human IgA ELISA Standard	1 vial/500 µl	4°C
400108	Immunoassay Buffer D Concentrate (5X)	2 vials/10 ml	4°C
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
400074	TMB Substrate Solution	1 vial/12 ml	4°C
10011355	HRP Stop Solution	1 vial/12 ml	RT
400012	96-Well Cover Sheet	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Human IgA ELISA Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

The stop solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (*e.g.* safety glasses, gloves, and lab coat) when using this material.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. An orbital microplate shaker
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
5. Materials used for **Sample Preparation** (see page 10)

INTRODUCTION

Background

Immunoglobulin A (IgA) is a member of the immunoglobulin superfamily of glycoproteins and is the most abundant antibody on human mucosal surfaces, such as the respiratory and gastrointestinal tracts.[1,2] It is produced by B cells and later secreted by activated B cells, called plasma cells. The IgA1 isotype predominates over IgA2 in human serum and is found primarily in a monomeric form.[3] Secretory IgA (SIgA), generated from dimeric IgA, is transported across the epithelial barrier to mucosal surfaces, where it acts as part of the first line of defense against pathogens.[3-6] In the intestine, SIgA binds to gut bacteria and prevents them from crossing the gut epithelium and, on the lumen side, prevents non-commensal bacteria from entering the intestine.[6,7] When administered into the nasal mucosa, monomeric IgA protects against lethality, weight loss, and inflammation in a mouse model of influenza.[8] IgA deficiency in humans is typically asymptomatic due to compensatory increases in IgG and IgM but may be related to allergy, autoimmune disorders, or recurrent infections and is associated with mild intestinal dysbiosis and an increase in pro-inflammatory gut bacteria.[7,9,10] IgA accumulates in small vessels and, in certain cases, the glomerulus in children with IgA vasculitis (IgAV), a disorder characterized by vascular inflammation, purpura, joint pain, gastrointestinal disturbances, and, in severe cases, glomerulonephritis.[11] Formulations containing IgA have been used as adjuncts in the treatment of bacterial infections and immunoglobulin substitution in immunodeficient patients. IgA-based monoclonal antibodies targeting tumor-associated antigens show promise in the treatment of cancer by inducing activation of FcαRI on immune cells.[12]

About This Assay

Cayman's Human IgA ELISA Kit is an immunometric assay (*i.e.* sandwich) that can be used for the quantification of the human IgA in biological fluids. The assay range is 0.78-50 ng/ml, with a lower limit of quantification of 0.78 ng/ml.

Principle Of This Assay

This immunometric assay is based on a double-antibody "sandwich" technique. Each well of the microwell plate supplied with the kit has been coated with a polyclonal antibody specific for human IgA. This antibody will bind any human IgA introduced into the well. A second polyclonal antibody conjugated to horseradish peroxide (HRP), which also recognizes human IgA, is added to the well forming a "sandwich". The "sandwich" is immobilized on the plate and the excess reagents are washed away. The concentration of human IgA is determined by measuring the enzymatic activity of HRP using the chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB). After a sufficient period, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of the color is directly proportional to the amount of bound antibody-HRP conjugate, which is proportional to the concentration of human IgA.

$$\text{Absorbance} \propto [\text{Anti-human IgA HRP}] \propto [\text{human IgA}]$$

A schematic of this process is shown below in Figure 1.

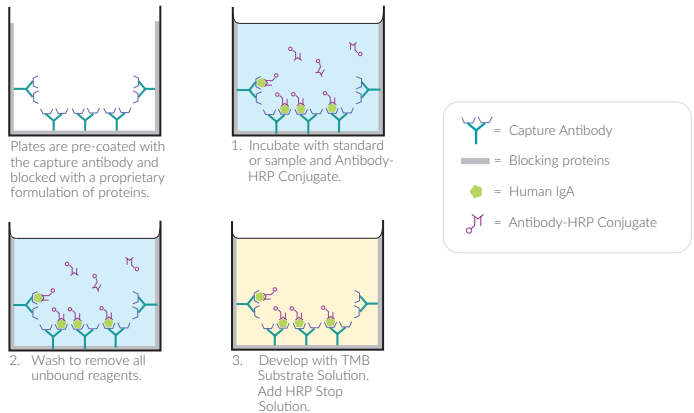

Figure 1. Schematic of the ELISA

Definition of Key Terms

Standard Curve: a plot of the absorbance values *versus* concentration of a series of wells containing various known amounts of analyte.

LLOD (Lower Limit of Detection): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

LLOQ (Lower Limit of Quantification): the lowest standard concentration in which absorbance (450 nm) – (1.64 x S.D.) is higher than the mean zero value of absorbance (450 nm) + (1.64 x S.D.).

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for approximately two months. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.*

1. **Assay Buffer (1X) Preparation**

   Dilute the contents of one vial of Immunoassay Buffer D Concentrate (5X) (Item No. 400108) with 40 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

2. **Wash Buffer (1X) Preparation**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20 (Item No. 400035). *NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.*

Sample Preparation

In general, biological fluids can be used directly in the assay following dilution in Assay Buffer (1X). Dilute plasma, serum, and saliva samples at least 10,000-20,000-fold in Assay Buffer (1X) to be in the range of the standard curve.

Testing for Interference and Minimal Dilution

This assay has been validated in plasma, serum, CSF, urine, and saliva. Other sample types should be tested for interference before embarking on a large number of sample measurements. To test for interference, dilute one or two test samples to obtain several dilutions for each sample and evaluate them in the assay. Calculate final IgA concentration for each dilution. The dilution where the final calculated human IgA concentration differs by 20% or less from the final concentration of previous dilution, is the minimum required dilution for that particular sample type.

Sample Matrix Properties

Parallelism

To assess parallelism, human plasma, serum, urine, and saliva samples were assayed at multiple dilutions using the Human IgA ELISA Kit. Concentrations were plotted as a function of sample dilution. The results are shown below.

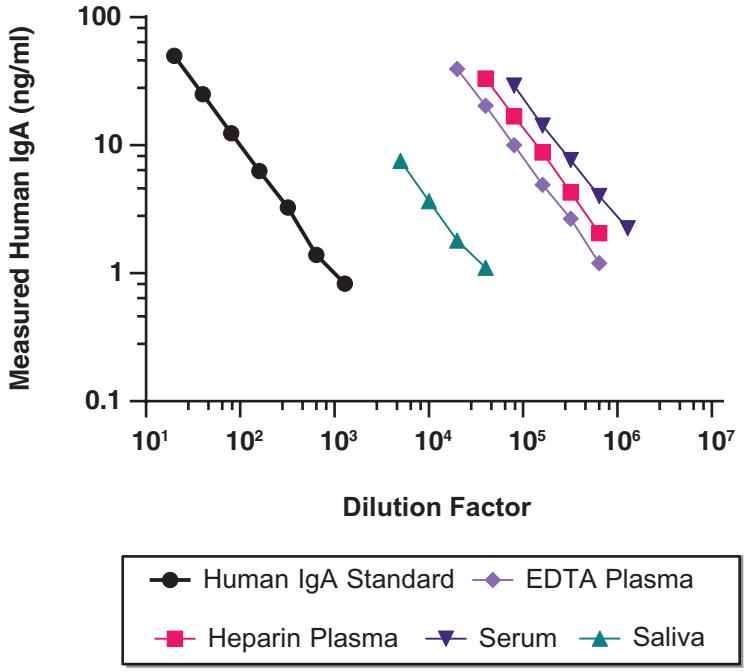

Figure 2. Parallelism of various matrices in the Human IgA ELISA Kit

Spike and Recovery

Human plasma (sodium citrate) and serum, both IgA depleted, and human urine were spiked with different amounts of human IgA, diluted with Assay Buffer (1X), and analyzed using the Human IgA ELISA Kit. The results are shown in Figures 3 and 4 below and on page 13.

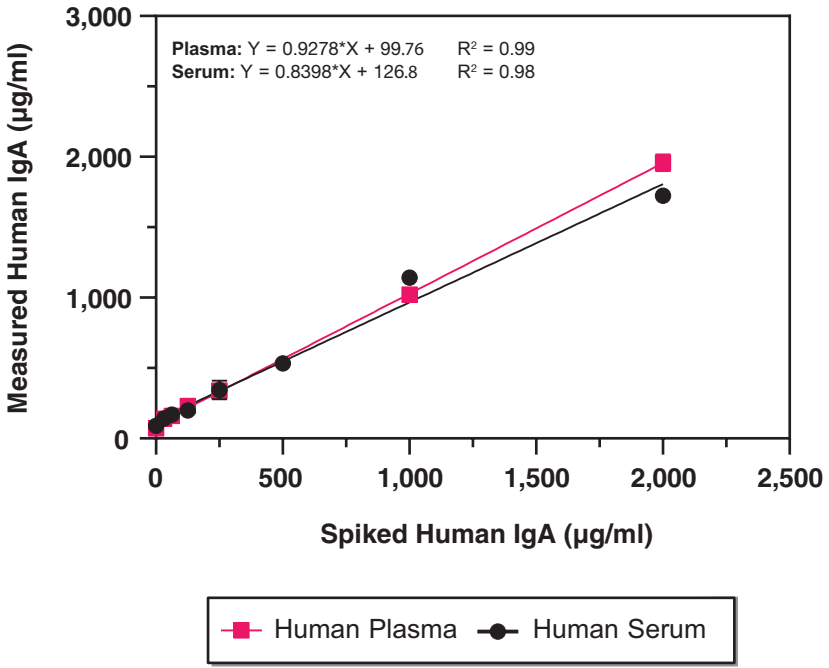

Figure 3. Spike and recovery of human IgA in plasma and serum

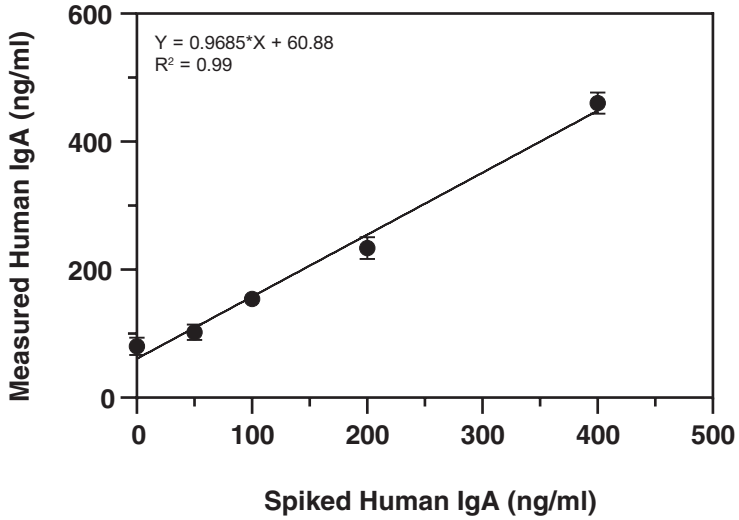

Figure 4. Spike and recovery of human IgA in urine

Linearity

Human plasma, serum, urine, and saliva were analyzed at multiple dilutions using the Human IgA ELISA Kit. The results are shown in Table 1, on page 15.

Dilution Factor	Measured IgA (ng/ml)	Linearity (%)
EDTA plasma, unspiked		
20,000	790,798	100
40,000	815,178	103
80,000	801,781	101
160,000	788,212	100
Heparin plasma, unspiked		
40,000	1,329,761	100
80,000	1,349,669	101
160,000	1,406,182	106
320,000	1,372,077	103
Serum, unspiked		
80,000	2,342,213	100
160,000	2,283,151	97
320,000	2,446,630	104
640,000	2,573,670	110
Urine, spiked with 400 ng/ml IgA		
10	442	100
20	464	105
40	475	107
Saliva, unspiked		
5,000	37,770	100
10,000	36,587	97
20,000	36,121	96
40,000	44,176	117

Table 1. Linearity in various matrices

*NOTE: Linearity has been calculated using the following formula: %Linearity = (Observed concentration value, dilution adjusted/First observed concentration value in the dilution series, dilution adjusted)*100*

Preparation of Assay-Specific Reagents

Human IgA ELISA Standard

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 950 µl Assay Buffer (1X) to tube #1 and 250 µl Assay Buffer (1X) into tubes #2-8. Equilibrate a pipette tip by repeatedly filling and expelling the tip with the Human IgA ELISA Standard (Item No. 400562) several times. Using the equilibrated pipette tip, transfer 50 µl of the standard into tube #1 and mix thoroughly. Serially dilute the standard by removing 250 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 250 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-7. Do not add any Human IgA ELISA Standard to tube #8. This tube is the zero standard or the background. These diluted standards should not be stored for more than two hours.

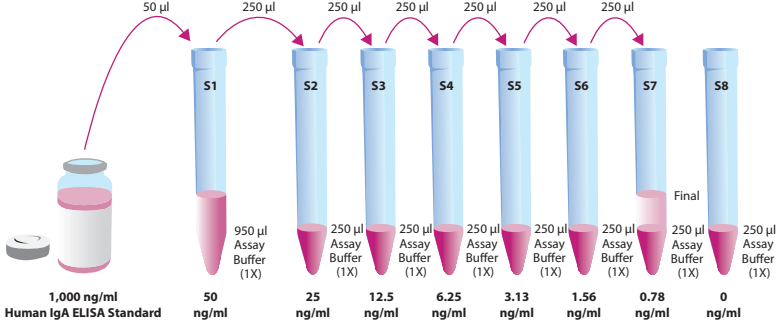

Figure 5. Preparation of the human IgA standards

Anti-Human IgA ELISA HRP Conjugate

Anti-Human IgA ELISA HRP Conjugate (Item No. 400561) is supplied as a concentrated (20X) stock solution of anti-human IgA antibody conjugated to HRP. On the day of the assay, bring the antibody-HRP conjugate to room temperature.

For a full plate, dilute 400 µl of the antibody-HRP conjugate into 7.6 ml of Assay Buffer (1X); for a half plate, dilute 200 µl of the antibody-HRP conjugate into 3.8 ml of Assay Buffer (1X) to make a 1X working solution. Do not prepare diluted antibody-HRP conjugate until immediately before use. Discard any unused antibody-HRP conjugate (1X). Store Anti-Human IgA ELISA HRP Conjugate (20X) stock solution at 4°C.

Plate Set Up

The 96-well plate(s) included with this kit must be pre-washed five times with Assay Buffer (1X) (~300 µl/well) prior to use in the ELISA. *NOTE: If you do not need to use all of the strips at once, place the unused strips back in the plate packet and store at 4°C. Be sure the packet is sealed with the desiccant inside.*

Each plate or set of strips must contain an eight point standard curve run in duplicate. *NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results.* Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 6, below. The user may vary the location and type of wells present as necessary for each particular experiment. We suggest recording the contents of each well on the template sheet provided (see page 29).

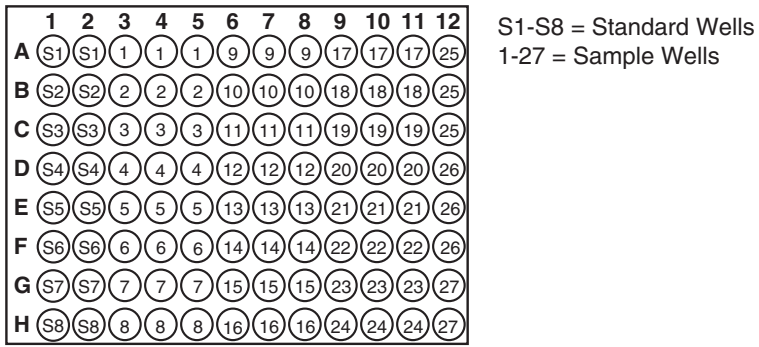

Figure 6. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Pre-Wash the Plate

Rinse the plate/plate strips five times with ~300 µl Wash Buffer (1X).

Addition of the Reagents

1. **Human IgA ELISA Standard and Samples**

   Pipette 50 µl of the Human IgA ELISA Standards or samples into the appropriate wells on the plate. Each sample should be assayed in duplicate, triplicate recommended.

2. **Anti-Human IgA ELISA HRP Conjugate**

   Prepare a 1X working solution of the Anti-Human IgA ELISA HRP Conjugate as described in the **Preparation of Assay-Specific Reagents** section. Add 50 µl of the Anti-Human IgA ELISA HRP Conjugate to all the wells.

Incubation of the Plate

1. Cover the plate with the 96-well Cover Sheet (Item No. 400012) and incubate for 60 minutes at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X). After the last wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer.
2. Add 100 µl of TMB Substrate Solution (Item No. 400074) to each well of the plate.
3. Cover the plate with the 96-well Cover Sheet. Optimum development is obtained by using an orbital shaker at room temperature for 15 minutes, protected from light.
4. DO NOT WASH THE PLATE. Add 100 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should remain colorless. *NOTE: The stop solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.*

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, *etc.*
2. Read the plate at a wavelength of 450 nm.

ANALYSIS

Many plate readers come with data reduction software that plots data automatically. Alternatively, a spreadsheet program can be used.

Calculations

Plotting the Standard Curve and Determining the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) *versus* concentration (log 10 x-axis) for standards (S1-S8) and fit the data with a four-parameter fit. Using the equation of the line, calculate the concentration of human IgA in each sample, making sure to correct for any sample dilution.

Performance Characteristics

Representative Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples. Variations in room temperature may cause variations in overall signal.

Human IgA ELISA Standards (ng/ml)	Absorbance (450 nm)	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
50	2.091	4.0	3.7
25	1.414	5.2	6.3
12.5	0.834	5.7	6.9
6.25	0.503	5.7	7.3
3.13	0.389	7.1	9.2
1.56	0.287	12.3	8.3
0.78	0.247	18.2	9.3
0	0.195	--	--

Table 2. Typical results
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve

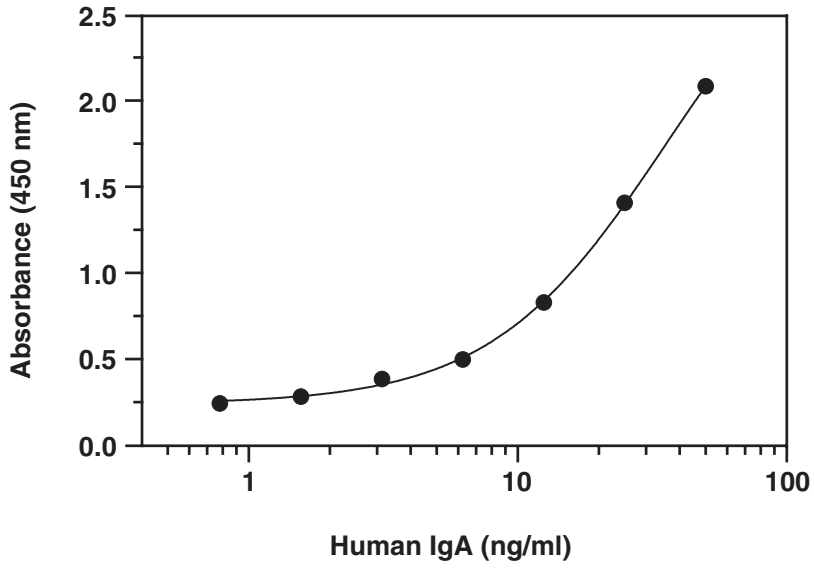

Figure 7. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of three matrix controls (human plasma, saliva, and urine) in a single assay.

Matrix Control (ng/ml)	%CV
951,018*	4.2
24,739**	4.3
81.3***	5.1

Table 3. Intra-assay precision
**Plasma **Saliva ***Urine*

Inter-assay precision was determined by analyzing replicates of three matrix controls (human plasma, saliva, and urine) in eight separate assays on different days.

Matrix Control (ng/ml)	%CV
715,120*	12
28,113**	13
58.4***	18

Table 4. Inter-assay precision
**Plasma **Saliva ***Urine*

Compound	Cross Reactivity
Human IgA	100%
Human IgE	<0.2%
Human IgG2	<0.04%
Human IgG3	<0.04%
Human IgG4	<0.04%
Human IgGM	<0.04%

Table 5. Cross reactivity of the Human IgA ELISA
NOTE: No cross reactivity of this assay was detected in rat, hamster, donkey, sheep, mouse, rhesus monkey, rabbit, or guinea pig serum diluted 1:1,000 with Assay Buffer (1X)

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High background wells (>0.150 O.D.)	A. Poor washing; ensure proper washing B. Exposure of background wells to standards or sample
Poor development (low signal) of standard curve	A. Trace organic contaminants in the water source B. Dilution error in preparing reagents
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present; determine minimal dilution for that sample type

References

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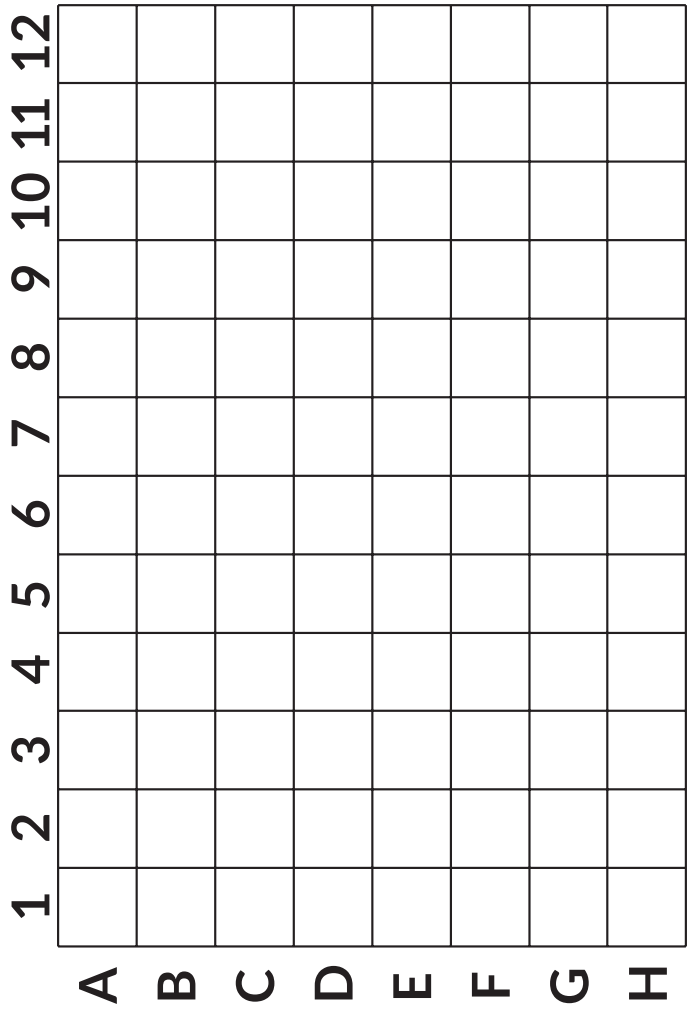
Procedure	Standards/Samples
Pre-Wash the Plate	Wash 5 x ~300 µl with Wash Buffer (1X)
Add standards/samples to plate	50 µl
Add antibody-HRP conjugate solution (1X)	50 µl
Incubate	Incubate plate for 60 minutes at RT on an orbital shaker
Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)
Add TMB Substrate Solution	100 µl
Develop	Seal plate and incubate for 15 minutes at RT on an orbital shaker protected from light
Do not wash. Add HRP Stop Solution	100 µl
Read	Read absorbance at 450 nm

Table 6. Assay summary



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.