Human IgE ELISA Kit

Item No. 502530

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/ Size	Storage Temperature
400661	Anti-Human IgE-biotin Conjugate	1 vial/400 µl	4°C
400662	Human IgE Standard	1 vial/100 µl	4°C
400663	Anti-Human IgE Strip Plate	1 plate	4°C
400664	Streptavidin Poly-HRP	1 vial/600 µl	4°C
400108	Immunoassay Buffer D Concentrate (5X)	2 vials/10 ml	4°C
400035	Polysorbate 20	1 vial/3 ml	RT
400074	TMB Substrate Solution	1 vial/12 ml	4°C
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
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 IgE 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 12)

INTRODUCTION

Background

Immunoglobulin E (IgE) is a member of the immunoglobulin superfamily of glycoproteins that plays a central role in type I hypersensitivity reactions and the immune response to parasites.[1-3] It is produced by B cells and later secreted by plasma cells and is the least abundant circulating immunoglobulin in human serum.[1,2] IgE consists of two light chains and two heavy chains, or ε chains, but lacks the flexible hinge region seen in IgD, IgG, and IgA.[3,4] IgE binds to type I Fcε receptors (FcεRIs) on the surface of mast cells, basophils, and antigen-presenting dendritic cells.[2] Multivalent antigen binding to IgE on the surface of mast cells induces IgE crosslinking and mast cell degranulation to initiate type I hypersensitivity reactions, including, but not limited to, systemic anaphylaxis, wheal and flare responses, allergic rhinitis, bronchial asthma, and food allergies. Serum levels of IgE are elevated in response to parasitic infection and IgE directly binds parasites to target the parasite for eosinophil degranulation-induced destruction. Salivary IgE levels are increased in patients with food allergies.[5] IgE-based monoclonal antibodies targeting tumor-associated antigens show promise in the treatment of cancer by inducing activation of FcεRIs on immune cells, reducing tumor growth, and increasing survival in immunocompetent animal models.[4]

About This Assay

Cayman's Human IgE ELISA Kit is an immunometric (*i.e.* sandwich) assay that can be used for the quantification of the human IgE in plasma, serum, CSF, urine, saliva, bronchoalveolar lavage fluid (BALF), and cell culture supernatants. The standard curve spans the range of 0-10 ng/ml, with a lower limit of detection of 0.005 ng/ml.

Principle Of This Assay

This immunometric assay is based on a double-antibody “sandwich” technique. Each well of the microtiter plate supplied with the kit has been coated with a polyclonal antibody specific to human IgE. If human IgE is present in the sample, it will bind to this immobilized polyclonal antibody in the first incubation step. A biotin-conjugated IgE detection antibody reagent added to the well binds to the human IgE, forming an immobilized “sandwich.” Excess reagents are washed away and a horseradish peroxide (HRP)-conjugated streptavidin reagent is added to the well. HRP-conjugated streptavidin binds to the biotin, allowing quantitation of the human IgE in the sample. The concentration of bound streptavidin 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-biotin conjugate, which is proportional to the concentration of the human IgE.

$$\text{Absorbance} \propto [\text{streptavidin HRP}] \propto [\text{anti-IgE biotin}] \propto [\text{IgE}]$$

A schematic of this process is shown in Figure 1, on page 9

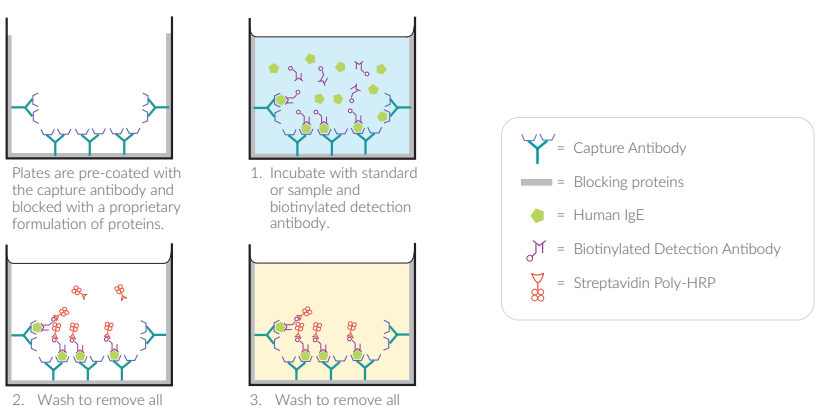


Figure 1. Schematic of the ELISA

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.

Lower Limit of Detection (LLOD): 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.

Lower Limit of Quantification (LLOQ): 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

This assay has been validated in plasma, serum, CSF, urine, saliva, BALF, and cell culture supernatants. Dilute the samples in Assay Buffer (1X) to be within the range of the standard curve. If testing undiluted cell culture medium samples, prepare the standard curve in the cell culture medium. Other sample types should be tested for interference to evaluate the need for a minimal dilution before embarking on a large number of sample measurements (see **Testing for Interference** below).

Testing for Interference

To test for interference, dilute one or two test samples to obtain several different dilutions for each sample. The dilution factor where the change in the final calculated human IgE concentration is consistent, differing by 20% or less than the previous dilution, is the minimum required dilution for that particular sample type.

Sample Matrix Properties

Parallelism

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

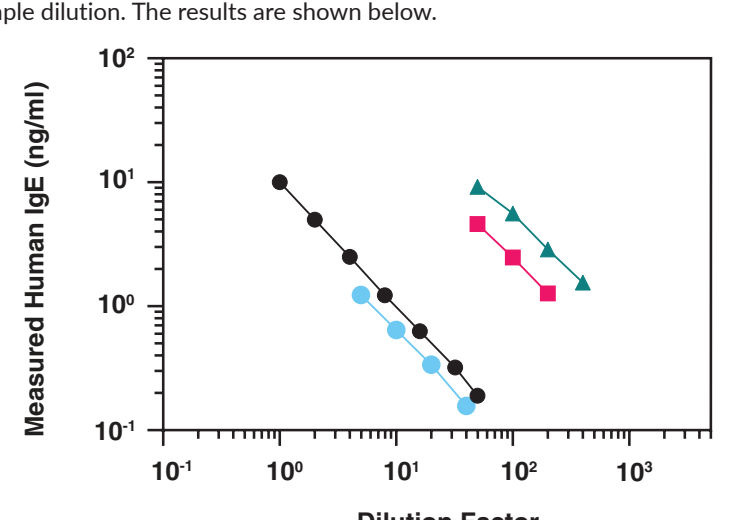


Figure 2. Parallelism of various matrices in the Human IgE ELISA Kit

Spike and Recovery

Human serum, human plasma, BALF, and cell culture media (DMEM+5%FBS) were spiked with human IgE, diluted with Assay Buffer (1X), and analyzed using the Human IgE ELISA Kit. The results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

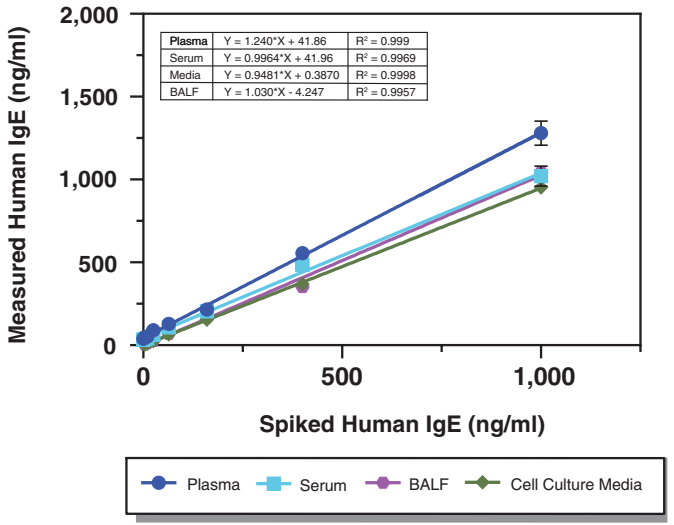


Figure 3. Spike and recovery of IgE in human serum

Linearity

Spiked human serum, urine, saliva, and HepG2 cell culture supernatant were analyzed at multiple dilutions using the Human IgE ELISA Kit. The results are shown in the table below.

Dilution Factor	Measured Concentration (ng/ml)	Linearity (%)
Serum (Spiked with 400 ng/ml Human IgE)		
40	426	100
80	412	96.7
160	458	108
Urine (Spiked with 50 ng/ml Human IgE)		
8	50.0	100
16	50.1	100
32	52.3	105
Saliva (Spiked with 50 ng/ml Human IgE)		
8	47.6	100
16	54.3	114
32	54.8	115
HepG2 Cell Supernatant (Spiked with 50 ng/ml Human IgE)		
8	48.9	100
16	55.0	112
32	54.0	110

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 IgE 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) to tubes #2-8. Equilibrate a pipette tip by repeatedly filling and expelling the tip with the Human IgE Standard (Item No. 400662) several times. Using the equilibrated pipette tip, transfer 50 µl of the bulk standard (200 ng/ml) to tube #1 and mix gently. 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 IgE Standard to tube #8. This tube is the background control. These diluted standards should not be stored for more than two hours.

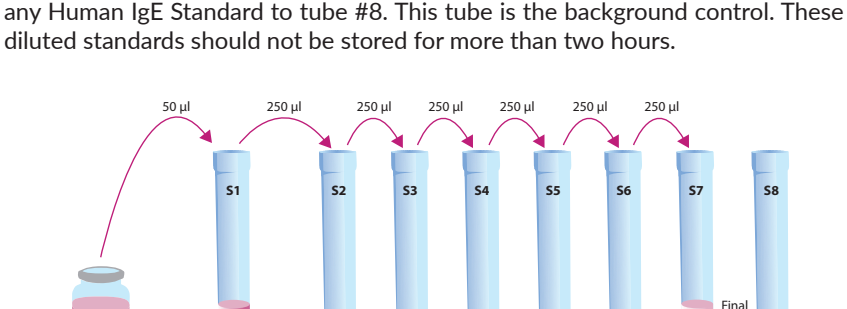


Figure 4. Preparation of the human IgE standards

Anti-Human IgE-biotin Conjugate (1X)

Anti-Human IgE-biotin Conjugate (Item No. 400661) is supplied as a concentrated (20X) stock solution of anti-human IgE antibody conjugated to biotin. At the time of assay, bring the Anti-Human IgE-biotin Conjugate to room temperature, then dilute 300 µl of the Anti-Human IgE-biotin Conjugate into 5.7 ml of Assay Buffer (1X) for a full plate or 150 µl of conjugate into 2.85 ml of Assay Buffer (1X) for a half plate to make a 1X working solution. Use this 1X solution within 4 hours.

Streptavidin Poly-HRP (1X)

Streptavidin Poly-HRP (Item No. 400664) is supplied as a concentrated (25X) stock solution of streptavidin conjugated to HRP. At the time of the assay, bring the Streptavidin Poly-HRP to room temperature.

For a full plate, dilute 480 µl of Streptavidin Poly-HRP into 11.52 ml of Assay Buffer (1X); for a half plate, dilute 240 µl of Streptavidin Poly-HRP into 5.76 ml of Assay Buffer (1X) to make a 1X working solution. Do not dilute Streptavidin Poly-HRP until immediately before use. Discard any unused Streptavidin Poly-HRP (1X).

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. *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, assaying the samples in triplicate is recommended.

A suggested plate format is shown in Figure 5, below. The user may vary the location and type of wells present as necessary for each particular experiment. It is suggested that the contents of each well on the template sheet provided (see page 29).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	1	1	1	9	9	9	17	17	17	25
B	S2	S2	2	2	2	10	10	10	18	18	18	25
C	S3	S3	3	3	3	11	11	11	19	19	19	25
D	S4	S4	4	4	4	12	12	12	20	20	20	26
E	S5	S5	5	5	5	13	13	13	21	21	21	26
F	S6	S6	6	6	6	14	14	14	22	22	22	26
G	S7	S7	7	7	7	15	15	15	23	23	23	27
H	S8	S8	8	8	8	16	16	16	24	24	24	27

S1-S8 = Standard Wells
1-27 = Sample Wells

Figure 5. 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.

Addition of the Reagents

1. **Human IgE Standard and Samples**

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

2. **Anti-Human IgE-Biotin Conjugate (1X)**

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

Incubation of the Plate

1. Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and incubate for 45 minutes at room temperature on an orbital shaker.

Addition of the Streptavidin Poly-HRP and Second Incubation

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. Prepare a 1X working solution of the Streptavidin Poly-HRP as described in the **Preparation of Assay-Specific Reagents** section.
3. Add 100 µl of the Streptavidin Poly-HRP (1X) working solution to each well of the plate.
4. Cover the plate with the 96-Well Cover Sheet and incubate for 30 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

Plot the Standard Curve and Determine the Sample Concentration

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

Performance Characteristics

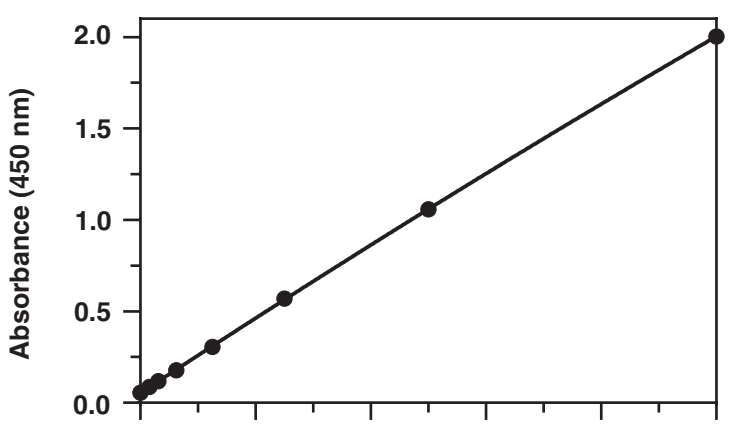
Representative Data

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 with your experiment. Do not use the data below to determine the values of your samples.

Absorbance at 450 nm

Human IgE Standards (ng/ml)	Blank-Corrected Absorbance	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
10	2.004	4.1	3.0
5	1.057	7.6	4.2
2.5	0.569	6.2	3.3
1.25	0.306	6.9	7.8
0.625	0.177	7.4	7.9
0.313	0.117	7.8	9.7
0.156	0.088	14.6	16.5
0	0.055	4.1	5.5

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



Assay Range = 0-10 ng/ml
Sensitivity (defined as LLOQ) = 0.156 ng/ml
Lower Limit of Detection (LLOD) = 0.005 ng/ml

The standards were diluted with Assay Buffer (1X).

Figure 6. 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.

Control	Measured IgE (ng/ml)	%CV
Control 1 (plasma)	736	4.6
Control 2 (spiked saliva)	50.5	7.2
Control 3 (spiked urine)	3.67	3.9

Table 3. Intra-assay precision

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

Control	Measured IgE (ng/ml)	%CV
Control 1 (plasma)	726	2.4
Control 2 (spiked saliva)	43.4	8.2
Control 3 (spiked urine)	5.16	14.0

Table 4. Inter-assay precision

Cross Reactivity:

Compound	Cross Reactivity
Human IgE	100%
Human IgG1	<0.002%
Human IgG2	<0.002%
Human IgG4	<0.002%
Human IgM	<0.002%
Human IgA	<0.002%

Table 5. Cross reactivity of the Human IgE ELISA
NOTE: No cross reactivity of this assay was detected in rat, mouse, rhesus monkey, or goat serum diluted 1:5 with Assay Buffer (1X) and tested for reactivity in this assay.

RESOURCES

Troubleshooting

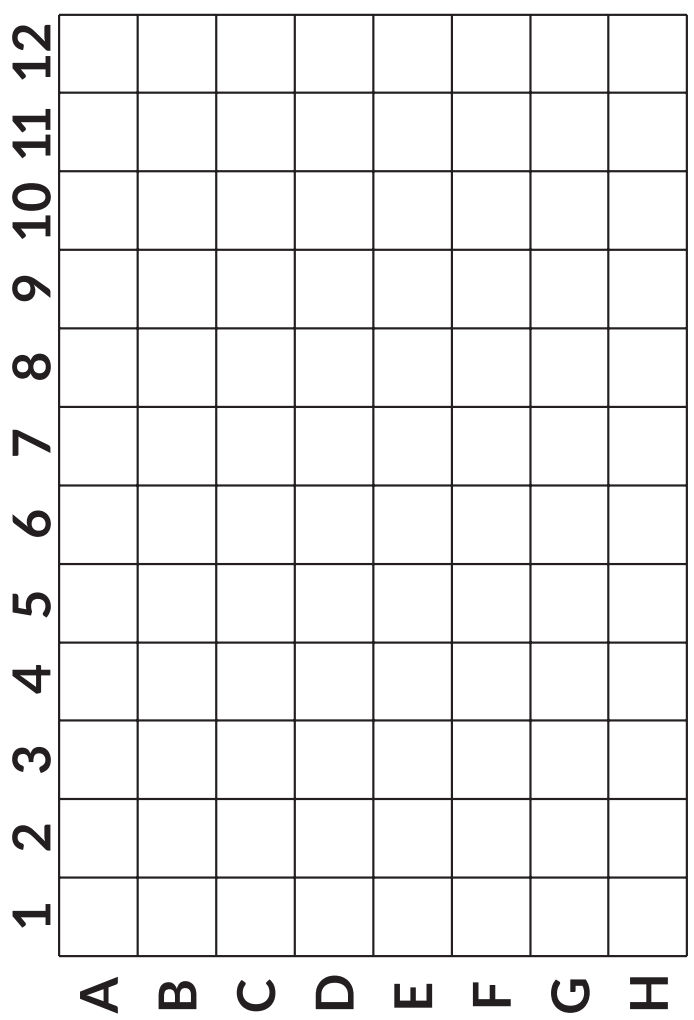
Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High background wells (>0.150 O.D.)	A. Poor washing B. Exposure of background wells to standards or samples
Poor development (low signal) of standard curve	A.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

References

1. Schroeder, H.W., Jr. and Cavicini, L. Structure and function of immunoglobulins. *J. Allergy Clin. Immunol.* **125(2 Suppl. 2)**, S41-S52 (2010).
2. Winter, W.E., Hardt, N.S., and Fuhrman, S. Immunoglobulin E: Importance in parasitic infections and hypersensitivity responses. *Arch. Pathol. Lab. Med.* **124(9)**, 1382-1385 (2000).
3. Gould, H.J., Sutton, B.J., Beavil, A.J., *et al.* The biology of IGE and the basis of allergic disease. *Annu. Rev. Immunol.* **21**, 579-628 (2003).
4. Sutton, B.J., Davies, A.M., Bax, H.J., *et al.* IgE antibodies: From structure to function and clinical translation. *Antibodies (Basel)* **8(1)**, 19 (2019).
5. Nunes, M.P.O., van Tilburg, M.F., Orlando, E., *et al.* Detection of serum and salivary IgE and IgG1 immunoglobulins specific for diagnosis of food allergy. *PLoS One* **14(4)**, e0214765 (2019).

Procedure	Standards/Samples
Add standards/samples to plate	50 µl
Apply antibody-biotin conjugate solution (1X)	50 µl
Incubate	Seal the plate and incubate for 45 minutes at room temperature on an orbital shaker
Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)
Apply Streptavidin Poly-HRP solution (1X)	100 µl
Incubate	Seal the plate and incubate for 30 minutes at room temperature on an orbital shaker
Aspirate	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)
Apply TMB Substrate Solution	100 µl
Develop	Seal the plate and incubate for 15 minutes at room temperature 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.