Mouse IgE ELISA Kit

Item No. 502850

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/ Size	Storage Temperature
401077	Anti-Mouse IgE ELISA Strip Plate	1 plate	4°C
401078	Anti-Mouse IgE ELISA Biotin Conjugate	1 vial/600 µl	4°C
401079	Mouse IgE ELISA Standard	1 vial/500 µl	4°C
400664	Streptavidin Poly-HRP	1 vial/600 µl	4°C
400108	Immunoassay Buffer D Concentrate (5X)	3 vials/10 ml	4°C
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
400062	Wash Buffer Concentrate (400X)	1 vial/5 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 Mouse 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 and plays a central role in type I hypersensitivity reactions and the immune response to parasites.[1-3] IgE production requires class switch recombination (CSR) from another Ig subclass.[4,5] In mice, class switching occurs only from IgG1 to IgE, whereas in humans it can occur *via* multiple IgG subclasses and IgA1, which leads to high variation in IgE responses between the two species.[5] IgE binds to the high-affinity IgE receptor, FcεRI, on the surface of mouse and human mast cells and basophils, as well as on the surface of additional human immune cell types. Transgenic mice engineered to have high basal levels of serum IgE display increased survival in a tumor model with no added benefit from active or passive immunotherapy.[6] Hyperproduction of IgE is associated with allergic diseases, and multivalent antigen binding to IgE on the surface of mast cells induces IgE cross-linking and mast cell degranulation to initiate type I hypersensitivity reactions, including systemic anaphylaxis, wheal and flare responses, allergic rhinitis, bronchial asthma, and food allergies, in humans.[2,7]

About This Assay

Cayman's Mouse IgE ELISA Kit is an immunometric (*i.e.* sandwich) assay that can be used for the quantification of mouse IgE in mouse plasma, serum, and urine, and cell culture media. The standard curve spans the range of 0-1,000 pg/ml, with a lower limit of detection (LLOD) of 4.8 pg/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 monoclonal antibody specific for mouse IgE. This antibody will bind any mouse IgE introduced into the well. A second polyclonal antibody conjugated to biotin, which also recognizes mouse IgE, is added to the well forming a “sandwich”. The “sandwich” is immobilized on the plate and the excess reagents are washed away. For quantification of the immobilized “sandwich”, streptavidin conjugated to horseradish peroxide (HRP) is then added to the wells. The streptavidin binds to the biotinylated “sandwich” and 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 mouse IgE.

Absorbance $\propto$ **[streptavidin HRP]** $\propto$ **[anti-IgE biotin]** $\propto$ **[mouse IgE]**

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

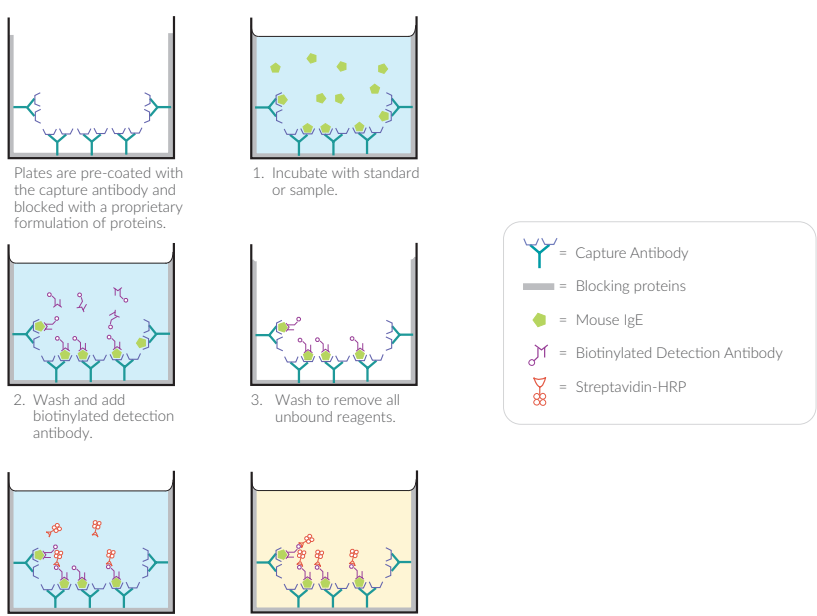


Figure 1. Schematic of the ELISA

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 at least two months. NOTE: It is normal for the concentrated buffer to contain crystalline salts. These will completely dissolve upon dilution with ultrapure water. 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.

1. **Assay Buffer Preparation**

   Dilute the contents of one vial of Immunoassay Buffer D Concentrate (Item No. 400108) with 40 ml of ultrapure water and add 50 µl of Polysorbate 20 (Item No. 400035). 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.

Sample Preparation

Sample Collection and Storage

This assay has been validated in mouse plasma, serum, and urine, and cell culture media. Dilute samples with Assay Buffer to be in the range of the standard curve. Typically, mouse plasma and serum can be diluted 50-10,000X to fall within the range of the standard curve. Mouse urine may need to be diluted at least two-fold. It is recommended that the values obtained from urine samples be standardized to creatinine levels using Cayman's Creatinine (urinary) Colorimetric Assay Kit (Item No. 500701), or a similar assay.

It is recommended to determine a minimum sample dilution before embarking on a large number of measurements. To determine the minimum sample dilution, dilute one or two test samples to obtain several different dilutions for each sample. The dilution factor where the change in the final calculated mouse IgE concentration is consistent, differing by 20% or less than the previous dilution, is the minimum required dilution for that particular sample type. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Sample Matrix Properties

Parallelism

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

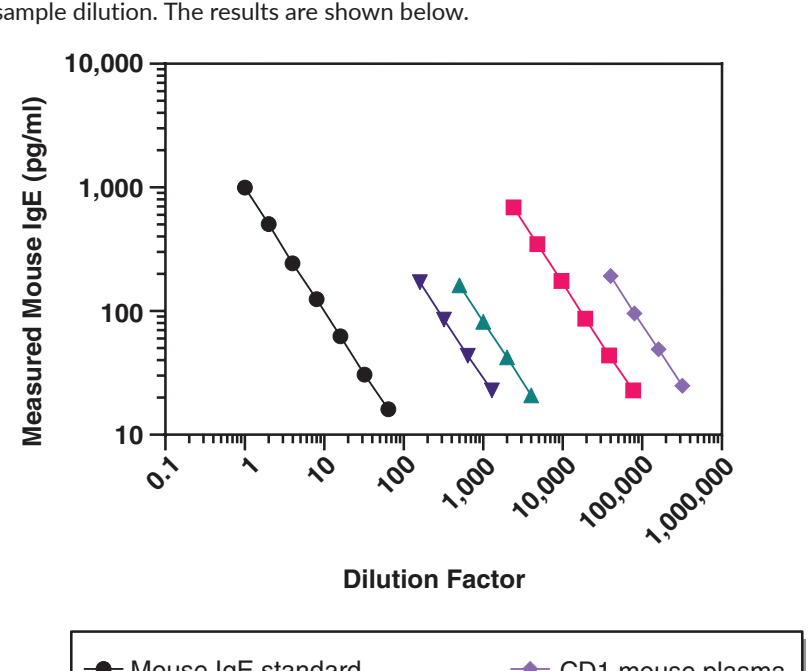


Figure 2. Parallelism of various matrices in the Mouse IgE ELISA Kit

Spike and Recovery

BALB/c mouse plasma was spiked with mouse IgE, diluted as described in the Sample Preparation section, and analyzed using the Mouse IgE ELISA Kit. The results are shown in Figure 3, below.

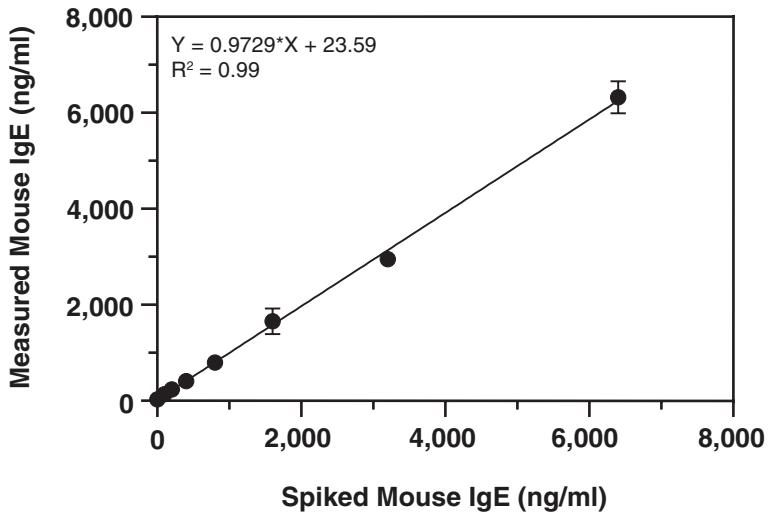


Figure 3. Spike and recovery of mouse IgE in BALB/c mouse plasma

Linearity

Mouse plasma, mouse urine, and DMEM complete medium were spiked with different amounts of mouse IgE and analyzed at multiple dilutions using the Mouse IgE ELISA Kit. The results are shown in the table below.

Dilution Factor	Measured Concentration (ng/ml)	Linearity (%)
BALB/c mouse plasma spiked with 6.4 µg/ml mouse IgE		
25,600	5,952	100
51,200	6,217	104
102,400	6,745	113
204,800	6,382	107
BALB/c mouse urine spiked with 50 ng/ml mouse IgE		
100	78.80	100
200	79.33	101
400	83.80	106
800	82.01	104
1,600	82.79	105
3,200	88.57	112
DMEM complete medium spiked with 50 ng/ml mouse IgE		
100	41.81	100
200	42.18	101
400	42.66	102
800	42.75	102
1,600	40.59	97.1

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

Mouse IgE Standard

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. On the day of the assay, bring the Assay Buffer to room temperature. Aliquot 960 µl Assay Buffer to tube #1 and 500 µl Assay Buffer to tubes #2-8. Equilibrate a pipette tip by repeatedly filling and expelling the tip with the Mouse IgE ELISA Standard (Item No. 401079) several times. Using the equilibrated pipette tip, transfer 40 µl of the standard to tube #1 and mix gently. Serially dilute the standard by removing 500 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 500 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-7. Do not add any Mouse IgE Standard to tube #8. This tube is the background control. These diluted standards should not be stored for more than two hours.

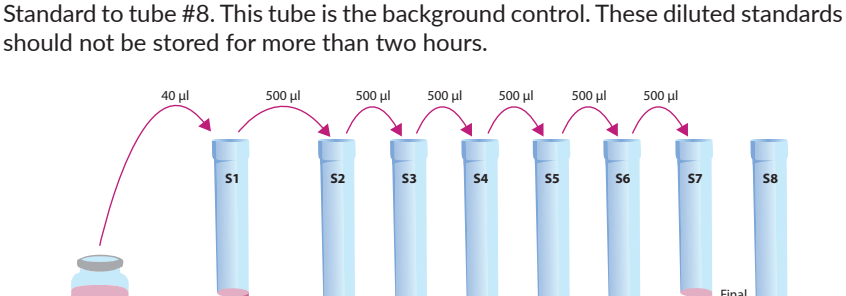


Figure 4. Preparation of the mouse IgE standards

Anti-Mouse IgE ELISA Biotin Conjugate

Anti-Mouse IgE ELISA Biotin Conjugate (Item No. 401078) is supplied as a concentrated (25X) stock solution of anti-mouse IgE antibody conjugated to biotin. On the day of the assay, bring the biotin conjugate to room temperature. For a full plate, dilute 500 µl of the Anti-Mouse IgE Biotin Conjugate with 12 ml of Assay Buffer; for a half plate, dilute 250 µl of the Streptavidin Poly-HRP with 6 ml of Assay Buffer to make a 1X mouse IgE ELISA biotin conjugate working solution. Use the diluted biotin conjugate within two hours of preparation.

Streptavidin Poly-HRP

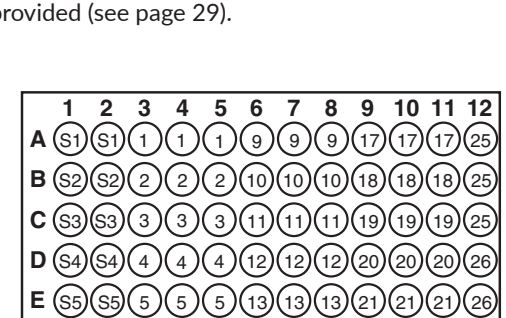
Streptavidin Poly-HRP (Item No. 400664) is supplied as a concentrated (25X) stock solution of streptavidin conjugated to HRP. On the day of the assay, bring the Streptavidin Poly-HRP to room temperature. For a full plate, dilute 500 µl of the Streptavidin Poly-HRP with 12 ml of Assay Buffer; for a half plate, dilute 250 µl of the Streptavidin Poly-HRP with 6 ml of Assay Buffer to make a 1X streptavidin poly-HRP conjugate working solution. Do not dilute Streptavidin Poly-HRP until immediately before use. Discard any unused Streptavidin Poly-HRP (1X). Store Streptavidin Poly-HRP (25X) stock solution at 4°C.

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 prior to adding the reagents. *NOTE: If you do not need to use all 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. Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed at least 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 be recorded on the template sheet provided (see page 29).



S1-S8 = Standard Wells
1-26 = 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 Standards and Samples and First Incubation

1. Pipette 100 µl of the Mouse IgE ELISA Standards or samples into the appropriate wells on the plate.
2. Cover the plate with the 96-Well Cover Sheet (Item No. 400012) and incubate for 60 minutes at room temperature on an orbital shaker.

Addition of the Anti-Mouse IgE Biotin Conjugate 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 Anti-Mouse IgE ELISA Biotin Conjugate as described in the **Preparation of Assay-Specific Reagents** section.
3. Add 100 µl of the Anti-Mouse IgE ELISA Biotin Conjugate (1X) working solution to each well of the plate.
4. Cover the plate with the 96-Well Cover Sheet and incubate for 60 minutes at room temperature on an orbital shaker.

Addition of the Streptavidin Poly-HRP Conjugate and Third 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 Streptavidin Poly-HRP as described in the **Preparation of Assay-Specific Reagents** section.
3. Add 100 µl of the Streptavidin-HRP Conjugate (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 30 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 mouse 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 each experiment. Do not use the data below to determine the values of your samples.

Mouse IgE ELISA Standards (pg/ml)	Absorbance (450 nm)	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
1,000	2.278	6.1	5.7
500	1.271	5.0	6.1
250	0.700	3.9	6.6
125	0.389	3.7	5.7
62.5	0.227	4.8	7.3
31.25	0152	5.8	6.5
15.625	0.129	7.3	15
0	0.0685	--	--

Table 2. Typical results

*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve

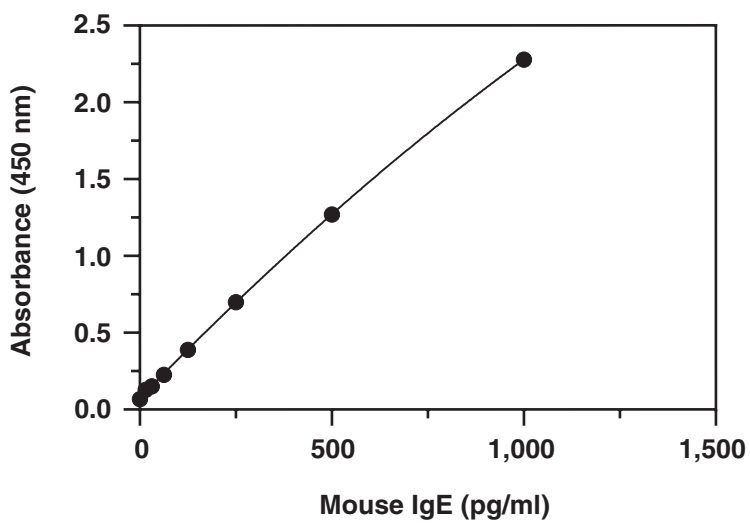


Figure 6. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of three matrix controls in a single assay.

Control	Measured IgE (ng/ml)	%CV
Control 1 (CD1 mouse plasma)	5,496	12.5
Control 2 (Non-Swiss albino mouse serum)	1,558	5.5
Control 3 (BALB/c mouse plasma)	27.9	3.6

Table 3. Intra-assay precision

Inter-assay precision was determined by analyzing replicates of three matrix controls in eight separate assays on different days.

Control	Measured IgE (ng/ml)	%CV
Control 1 (CD1 mouse plasma)	6,512	10.7
Control 2 (Non-Swiss albino mouse serum)	1,516	5.5
Control 3 (BALB/c mouse plasma)	24.8	3.5

Table 4. Inter-assay precision

Cross Reactivity:

Compound	Cross Reactivity
Mouse IgE	100%
Mouse IgA	0.02%
Mouse IgM	0.02%
Mouse IgG1	0.02%
Mouse IgG2a	0.02%
Mouse IgG2b	0.02%
Mouse IgG2c	0.02%
Mouse IgG3	0.02%

Table 5. Cross reactivity of the Mouse IgE ELISA
NOTE: No cross reactivity was detected in donkey, bovine, rat, rhesus monkey, goat, rabbit, guinea pig, sheep, or hamster serum diluted 1:5 with Assay Buffer.

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of replicates	A. Trace organic contaminants in the water 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 samples
Poor development (low signal) of standard curve	A. Trace organic contaminants in the water source B. Dilution error in preparing reagents C. Streptavidin Poly-HRP was not diluted immediately prior to use
Poor development (low signal) of samples	Specific sample does not contain IgE antibodies or is too dilute
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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4. Gould, H.J. and Sutton, B.J. IgE in allergy and asthma today. *Immunology* **8**, 205-217 (2008).
5. Gould, H.J. and Ramadani, F. IgE responses in mouse and man and the persistence of IgE memory. *Trends Immunol.* **36(1)**, 40-48 (2015).
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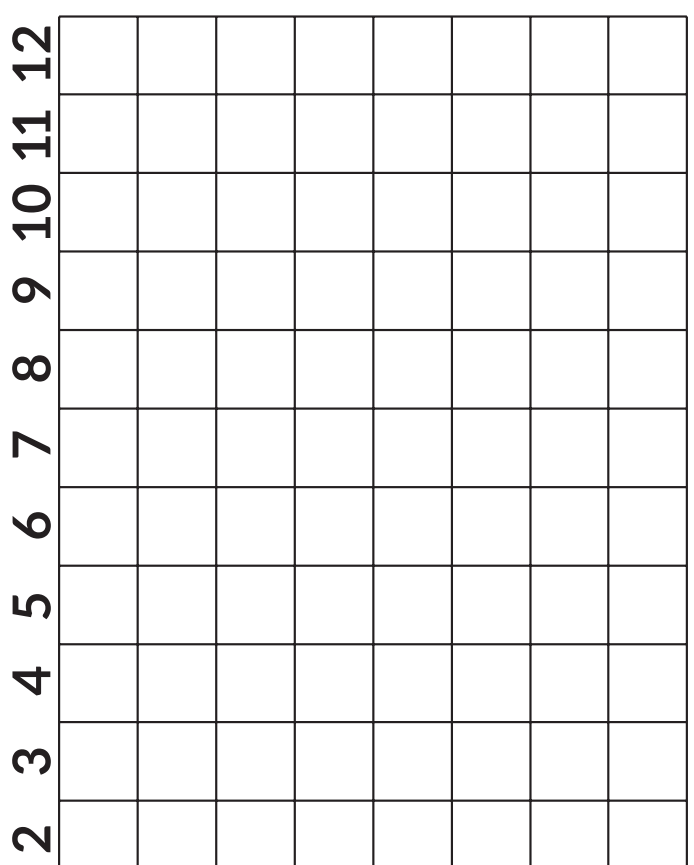
7. Gusareva, E.S., Blažková, H., Kosařová, M., *et al.* Mouse to human comparative genetics reveals a novel immunoglobulin E-controlling locus on Hsa8q12. *Immunogenetics* **61**, 15-25 (2009).

Assay Summary

Procedure	Standard/Samples
Mix all reagents gently	--
Add standards/samples to plate	100 µl
Incubate	Seal the plate and incubate for 1 hour at room temperature on an orbital shaker
Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)
Add Anti-Mouse IgE ELISA Biotin Conjugate (1X) working solution	100 µl
Incubate	Seal the plate and incubate for 1 hour at room temperature on an orbital shaker
Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)
Add Streptavidin Poly-HRP conjugate (1X) working solution	100 µl
Incubate	Seal the plate and incubate for 30 minutes at room temperature on an orbital shaker
Wash	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)
Add TMB	100 µl
Develop	Seal plate and incubate for 30 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

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