Mouse IgG ELISA Kit

Item No. 501240

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
401241	Mouse IgG Assay HRP-Conjugate	2 vials/1.5 ml
400009	Goat Anti-Mouse IgG Precoated 96-Well Strip Plate	1 plate
401244	IgG (mouse) ELISA Standard	1 vial/0.25 ml
400060	ELISA Buffer Concentrate (10X)	2 vials/10 ml
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml
400035	Polysorbate 20	1 vial/3 ml
400074	TMB Substrate Solution	1 vial/12 ml
10011355	HRP Stop Solution	1 vial/12 ml
400012	96-Well Cover Sheet	3 covers

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's Mouse IgG 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 at 4°C 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. Adjustable pipettes and a repeating pipettor.
3. 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).*
4. Materials used for **Sample Preparation** (see page 9).

INTRODUCTION

Background

Immunoglobulins are heterodimeric proteins composed of two heavy and two light chains. There are five classes of immunoglobulins found in mammals: IgA, IgD, IgE, IgG and IgM, which differ in their corresponding types of heavy chains, designated as α, γ, ε, δ, and μ. The various classes of immunoglobulins have different physiological functions.[1,2]

IgG is the most abundant immunoglobulin of all five, present in blood and extracellular fluid. It provides the majority of antibody-based immunity against invading pathogens. IgG also plays a significant role in the pathology of auto-immune diseases. It is the only immunoglobulin that can cross the placenta to give passive immunity to the fetus. The common IgG isotypes have a molecular weight of 150 kD. Under reducing condition, each heavy chain is about 50 kD and each light chain is about 25 kD.[1,2]

Mice provide a widely used research model for studies of the immune system, various diseases and drug development. Mouse monoclonal antibodies have become an important tool in biochemistry, molecular biology and medicine, and most mouse monoclonal antibodies are of the IgG isotype. There are multiple applications for a sensitive assay to accurately quantify the concentration of mouse IgG in complex sample matrices.

About This Assay

Cayman's Mouse IgG ELISA Kit is a sandwich assay which can be used to measure IgG in mouse plasma, mouse serum, cell culture supernatants and ascites without prior sample purification. The standard curve spans the range of 1.6-100 ng/ml with a lower limit of quantification (LLOQ) of 1.6 ng/ml. The reported measured levels of IgG in mouse serum range between 2 and 22 mg/ml.[3]

Description of Immunometric ELISAs

This immunometric assay is based on a double-antibody 'sandwich' technique. Each well of the microwell plate supplied in the kit has been coated with an antibody specific for mouse IgG. This antibody will bind any mouse IgG introduced into the well. A second antibody recognizing a different epitope of mouse IgG (Antibody/HRP Conjugate) is added to the well. This allows the two antibodies to form a 'sandwich' by binding two different epitopes on the mouse IgG molecule. The 'sandwiches' are immobilized on the plate so the excess reagents may be washed away. The Antibody/HRP Conjugate is labeled with HRP, allowing quantitation of the mouse IgG. Addition of HRP Substrate TMB, followed by Stop Solution produces a yellow colored product which can be measured spectrophotometrically. The intensity of the color is directly proportional to the amount of bound Antibody/HRP Conjugate, which is proportional to the concentration of IgG.

$$\text{Absorbance} \propto [\text{Anti-mouse IgG/HRP}] \propto [\text{mouse IgG}]$$

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

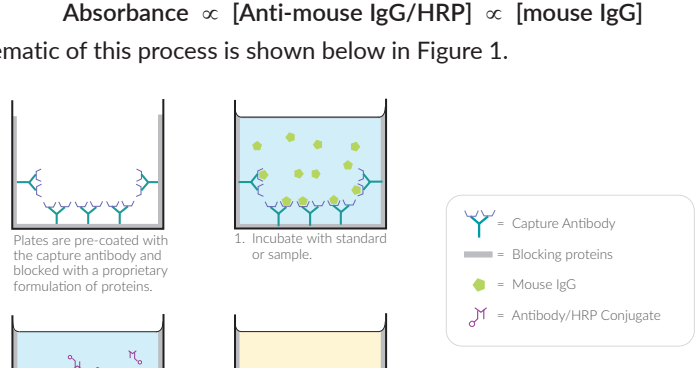


Figure 1. Schematic of the Immunometric ELISA

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.

Cross Reactivity: numerical representation of the relative reactivity of this assay towards structurally related molecules as compared to the primary analyte of interest. Biomolecules that possess similar epitopes to the analyte can compete with the assay tracer for binding to the primary antibody. Substances that are superior to the analyte in displacing the tracer result in a cross reactivity that is greater than 100%. Substances that are inferior to the primary analyte in displacing the tracer result in a cross reactivity that is less than 100%. Cross reactivity is calculated by comparing the mid-point (50% B/B_0) value of the tested molecule to the mid-point (50% B/B_0) value of the primary analyte when each is measured in assay buffer using the following formula:

$$\text{\% Cross Reactivity} = \left[\frac{\text{50\% } B/B_0 \text{ value for the primary analyte}}{\text{50\% } B/B_0 \text{ value for the potential cross reactant}} \right] \text{x } 100\%$$

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for about two months.

1. **ELISA Buffer Preparation**

   Dilute the contents of one vial of ELISA Buffer Concentrate (10X) (Item No. 400060) with 90 ml of water. Be certain to rinse the vial to remove any salts that may have precipitated. *NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.*

2. **Wash Buffer Preparation**

   5 ml vial Wash Buffer Concentrate (400X) (Item No. 400062): Dilute to a total volume of 2 L with water 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 this assay, mouse serum or plasma (prepared using heparin or EDTA as the anticoagulant), cell culture supernatants and mouse ascites can be measured directly following dilution in ELISA Buffer.

Preparation of Assay-Specific Reagents

IgG (mouse) ELISA Standard

The concentration of the supplied mouse IgG standard solution (Item No. 401244) is 10 µg/ml.

To prepare the standard for use in the ELISA: Obtain eight clean test tubes and label them, #1 through #8. Aliquot 990 µl of ELISA Buffer into tube #1 and 500 µl of ELISA Buffer into tubes #2-8. Transfer 10 µl of the mouse IgG (10 µg/ml) to tube #1. Serially dilute the standard by removing 500 µl from tube #1 and placing into tube #2. Mix gently. Next, remove 500 µl from tube #2 and place into tube #3; mix gently. Repeat this process for tubes #4-7. Do not add any mouse IgG to tube #8. This tube is the zero-point vial, the lowest point on the standard curve.

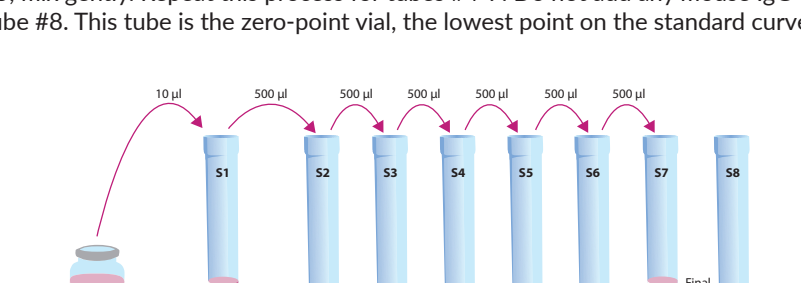


Figure 2. Preparation of the IgG standards

Mouse IgG Assay HRP-Conjugate

This reagent is supplied as a concentrated (10X) stock solution of goat anti-mouse IgG polyclonal antibody conjugated to HRP. On the day of the assay, prepare a 1X working solution by adding 1.2 ml of the Mouse IgG Assay HRP-Conjugate (Item No. 401241) to 10.8 ml ELISA Buffer (12 ml total). ***Use HRP-Conjugate diluted in ELISA Buffer immediately.*** In the event that more than one experiment is performed with this kit, two vials of stock solution have been provided to produce an additional 12 ml of the working solution.

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, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 3, below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 15, for more details). We suggest you record the contents of each well on the template sheet provided (see page 22).

	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 1-8
1-27 - Sample Wells

Figure 3. 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 Standards and Samples and First Incubation

1. Add 100 µl of the standards or diluted sample to the appropriate wells on the plate. Each sample should be assayed in duplicate, triplicate recommended.
2. Cover the plate with the 96-Well Cover Sheet (Item No. 400012). Incubate for one hour at room temperature on an orbital shaker.

Addition of HRP-Conjugate and Second Incubation

1. Empty the wells and rinse four times with Wash Buffer. Each well should be completely filled with Wash Buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbent paper to remove the residual Wash Buffer.
2. Add 100 µl of the HRP-Conjugate working solution to each well of the plate.
3. Cover the plate with plastic film and incubate for one hour at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse four times with Wash Buffer. Each well should be completely filled with Wash Buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. 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 plastic film and incubate for 30 minutes at room temperature on an orbital shaker.
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 (linear x-axis) for standards (S1-S7) and fit the data with a linear equation.

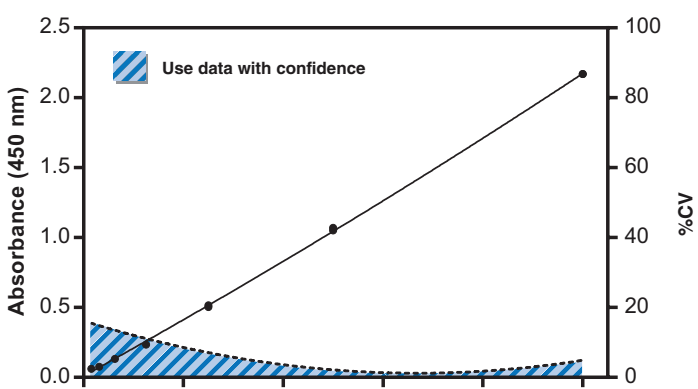
Performance Characteristics

Sample 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. Your results could differ substantially. Development of the plate for 30 minutes typically results in an absorbance of >1.0 O.D. units for the 100 ng/ml standard.

Mouse IgG (ng/ml)	Absorbance	
100	2.170	2.170
50	1.051	1.07
25	0.515	0.504
12.5	0.234	0.224
6.3	0.132	0.13
3.2	0.076	0.076
1.6	0.061	0.061
0	0.034	0.035

Table 1. Typical results



Assay Range = 1.6-100 ng/ml
LLOQ = 1.6 ng/ml

The lower limit of quantitation (LLOQ) is defined as the lowest standard concentration in which O.D. – (1.64 x S.D.) is higher than the blank value of O.D. + (1.64 x S.D.).The standard was diluted with ELISA Buffer.

Mouse IgG Standard curve
Mouse IgG Intra-assay variation

Figure 4. Typical standard curve

Precision:

The intra-assay CVs have been determined at multiple points on the standard curve. These data are summarized in the graph on page 17 and in the table below.

Dose (ng/ml)	%CV* Intra-assay variation
100	4.38
50	4.88
25	5.46
12.5	7.22
6.3	13.95
3.2	13.65
1.6	19.59

Table 2. Intra-assay variation

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

IgG (ng/ml)	Mean of O.D.	Standard Deviation (S.D.)	O.D. - (1.64 x S.D.)
100	2.348	0.048	2.267
50	1.256	0.055	1.166
25	0.636	0.032	0.584
12.5	0.319	0.001	0.317
6.3	0.184	0.007	0.172
3.2	0.100	0.005	0.092
1.6	0.079	0.001	0.078
0.8	0.060	0.003	0.055
0	0.050	0.007	0.062

*O.D. + (1.64 x S.D.)

Table 3. Determination of LLOQ

The lower limit of quantitation (LLOQ) is defined as the lowest standard concentration in which O.D. - (1.64 x S.D.) is higher than the blank value of O.D. + (1.64 x S.D.). The LLOQ is 1.6 ng/ml.

Sample	Average Value (µg/ml)	%CV
Mouse plasma	7,005	6.73
Mouse serum	4,791	8.43
Cell culture supernatant	57.4	7.71
Mouse ascites	1,081	10.03

Table 4. Sample Inter-assay validation. Each sample was tested a total of eight times using different sets of standards on different plates and on different days.

Compound	Cross Reactivity
Mouse IgG	100%
Mouse IgE	3.69%
Mouse IgA	0.20%
Mouse IgM	0.01%

Table 5. Cross Reactivity

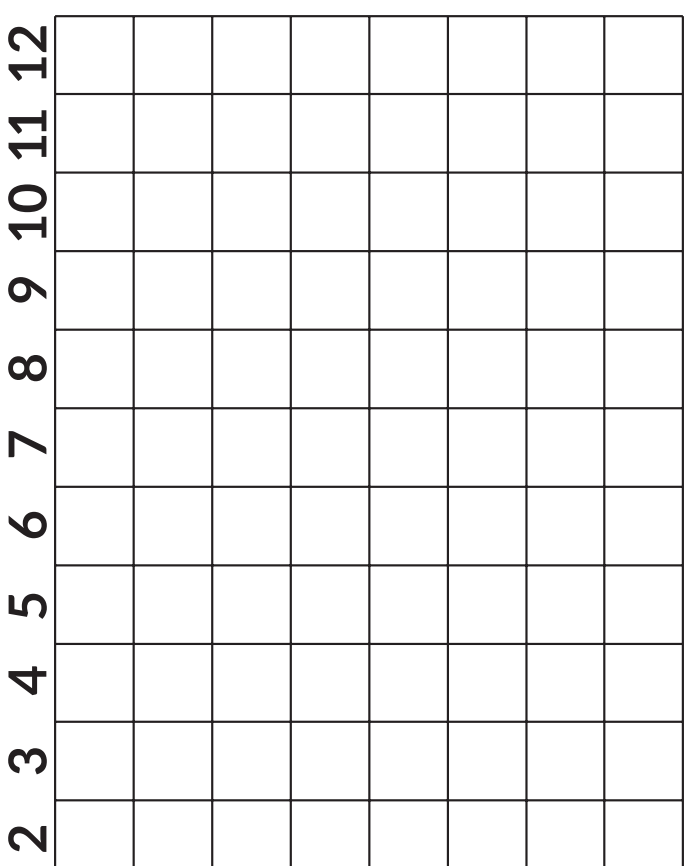
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique	A. Replace activated carbon filter or change source of UltraPure water
Poor development (low signal) of standard curve	A. Plate required more development time B. Standard was diluted incorrectly C. Standard is degraded	

References

1. Janeway, C., Travers, P., Walport, M., *et al.* Antigen recognition by B-cell and T-cell receptors, Chapter 3, *in* Immunobiology. 6th, Garland Science Publishing, New York, NY, 103-107 (2004).
2. Schroeder, H.W., Jr. and Cavicini, L. Structure and function of immunoglobulins. *J. Allergy Clin. Immunol.* **125(2 Suppl 2)**, S41-S52 (2010).
3. Klein-Schneegans, A.-S., Kuntz, L., Fonteneau, P., *et al.* Serum concentrations of IgM, IgG1, IgG2b, IgG3 and IgA in C57BL/6 mice and their congenics at the *lpr* (lymphoproliferation) locus. *J. Autoimmun.* **2(6)**, 869-875 (1989).



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.

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