Mouse IgG1 ELISA Kit

Item No. 502710

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/ Size	Storage Temperature
400697	Anti-Mouse IgG1 ELISA HRP Conjugate	1 vial/0.5 ml	4°C
400698	Mouse IgG1 ELISA Standard	1 vial/0.5 ml	4°C
400699	Anti-Mouse Ig ELISA Strip Plate	1 plate	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 IgG1 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 G (IgG) is a member of the immunoglobulin superfamily of glycoproteins that plays a central role in the adaptive immune response and is the most abundant circulating antibody in human and mouse sera.[1,2] It is produced by B cells following IgM class-switching in response to infection and later secreted by plasma cells.[1,3,4] IgG is involved in numerous humoral host defense responses, including antibody-dependent cell-mediated cytotoxicity (ADCC), toxin neutralization, and pathogen opsonization.[3] IgG1 is one of four IgG isotypes found in mice and its production is driven by Th2-stimulated immune responses.[5,6] IgG1 neutralizes toxins and activates the inhibitory FcγRIIB receptor but does not trigger FcγR-mediated effector functions.

About This Assay

Cayman's Mouse IgG1 ELISA Kit is an immunometric (*i.e.* sandwich) assay that can be used for the quantification of mouse IgG1 in mouse plasma, serum, and urine and cell culture media. The standard curve spans the range of 1.56-100 ng/ml with a lower limit of detection (LLOD) of 0.23 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 mouse immunoglobulin. This antibody will bind any mouse IgG1 introduced into the well. A second polyclonal antibody conjugated to horseradish peroxidase (HRP), which recognizes IgG1 specifically, 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 mouse IgG1 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 HRP conjugate, which is proportional to the concentration of mouse IgG1.

$$\text{Absorbance} \propto [\text{anti-IgG1-HRP}] \propto [\text{IgG1}]$$

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

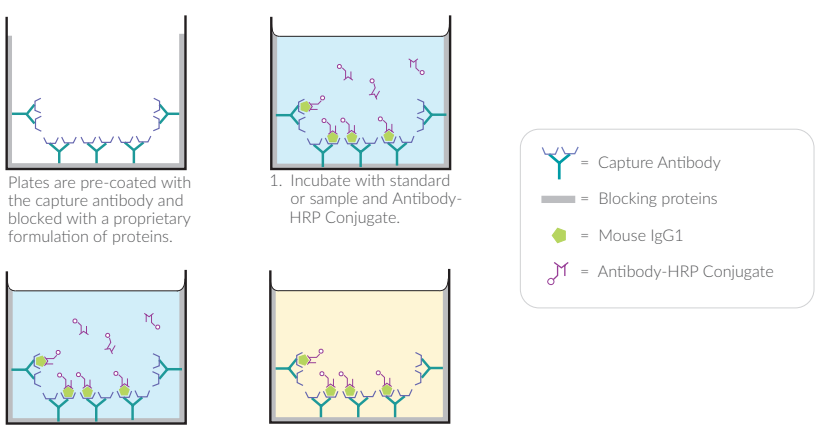


Figure 1. Schematic of the ELISA

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. These will completely dissolve upon dilution with ultrapure water.

1. **Immunoassay Buffer D (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

Sample Collection and Storage

This assay has been validated in mouse plasma, serum, and urine and cell culture media. Typically, plasma and serum samples should be diluted between 10,000 and 100,000-fold with Immunoassay Buffer D (1X) for the values to fall within the range of the standard curve. Mouse urine may need to be diluted at least 10-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 a minimum sample dilution, dilute one or two test samples to obtain several different dilutions for each sample. The dilution where the final calculated mouse IgG1 concentration (dilution adjusted) differs by 20% or less from the result from previous dilution, is the minimum required dilution for that particular sample.

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, serum, and urine were assayed at multiple dilutions using the Mouse IgG1 ELISA Kit. Concentrations were plotted as a function of sample dilution. The results are shown below.

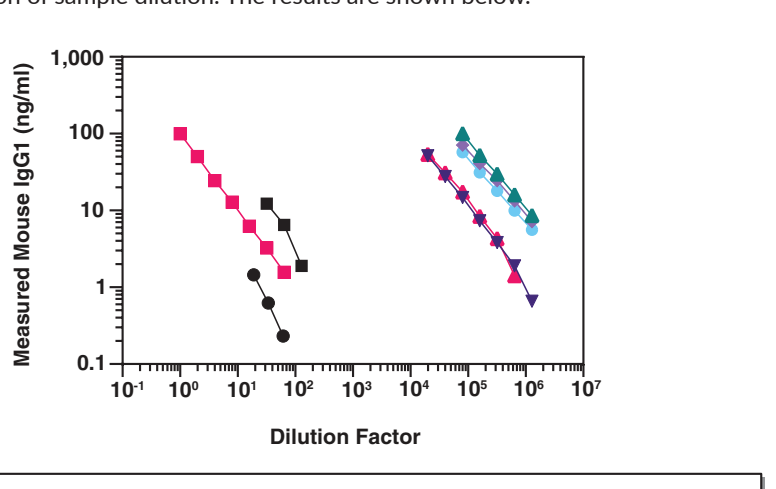


Figure 2. Parallelism of various matrices in the Mouse IgG1 ELISA Kit

Spike and Recovery

IgG-depleted mouse plasma and serum were spiked with mouse IgG1, diluted with Immunoassay Buffer D (1X), and analyzed using the Mouse IgG1 ELISA Kit. The results are shown below.

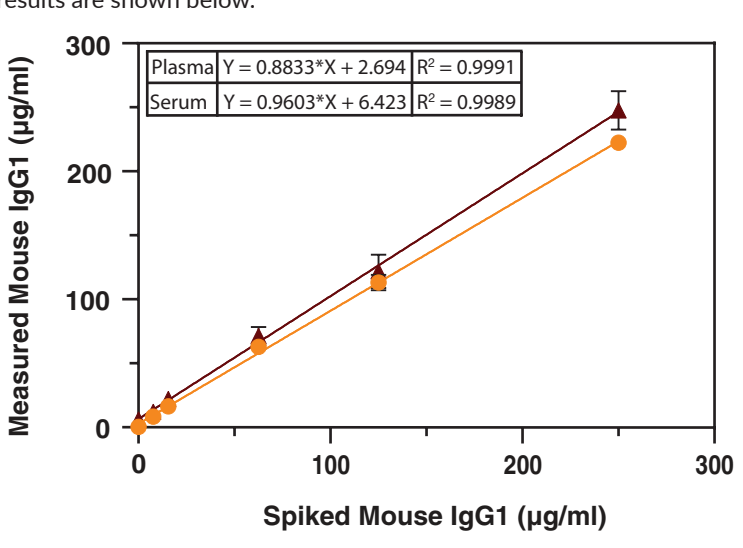


Figure 3. Spike and recovery of mouse IgG1 in mouse plasma and serum

Linearity

Mouse plasma and serum (both IgG-depleted using a protein G column), mouse urine, and cell culture medium (RPMI containing 3% FBS and phenol red) were spiked with mouse IgG1 and analyzed at multiple dilutions using the Mouse IgG1 ELISA Kit. The results are shown in the table below.

Dilution	Measured Concentration (µg/ml)	Dilutional Linearity (%)
Plasma, spiked with 250 µg/ml IgG1		
1:5,000	223	100
1:10,000	223	100
1:20,000	224	100
Serum, spiked with 250 µg/ml IgG1		
1:5,000	247	100
1:10,000	226	91.5
1:20,000	260	106
Cell culture medium, spiked with 1 µg/ml IgG1		
1:32	0.976	100
1:64	1.118	115
1:128	1.138	117
Urine, spiked with 0.250 µg/ml IgG1		
1:8	0.308	100
1:16	0.291	94.5

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 IgG1 ELISA Standard

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 950 µl Immunoassay Buffer D (1X) to tube #1 and 500 µl Immunoassay Buffer D (1X) to tubes #2-8. Equilibrate a pipette tip by repeatedly filling and expelling the tip with the Mouse IgG1 ELISA Standard (Item No. 400698) several times. Transfer 50 µl of the stock standard (2,000 ng/ml) to tube #1 and mix thoroughly. 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 IgG1 ELISA Standard to tube #8. This tube is the background control. These diluted standards should not be stored for more than eight hours.

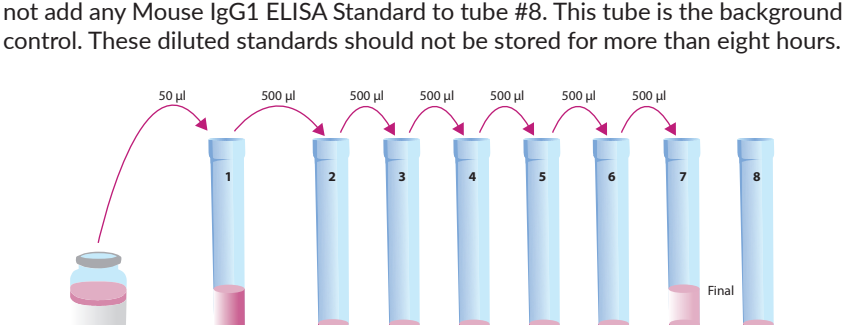


Figure 4. Preparation of the mouse IgG1 standards

Anti-Mouse IgG1 ELISA HRP Conjugate (1X)

The Anti-Mouse IgG1 ELISA HRP Conjugate (Item No. 400697) is supplied as a concentrated (20X) stock solution of anti-mouse IgG1 antibody conjugated to HRP.

For a full plate, dilute 300 µl of the HRP conjugate into 5.7 ml of Immunoassay Buffer D (1X); for a half plate, dilute 150 µl of the HRP conjugate into 2.85 ml of Immunoassay Buffer D (1X) to make a 1X working solution. The HRP Conjugate (1X) will be stable for 2 hours. Discard any unused HRP conjugate (1X).

Plate Set Up

The 96-well plate(s) included with this kit must be pre-washed five times with Wash Buffer (1X) (300 µl/well) prior to use in the ELISA. *NOTE: Do not store strips after pre-washing. If you do not need to use all the strips at once, place the unused, unwashed 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.

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).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	1	1	9	9	17	17	25	25	33	33
B	S2	S2	2	2	10	10	18	18	26	26	34	34
C	S3	S3	3	3	11	11	19	19	27	27	35	35
D	S4	S4	4	4	12	12	20	20	28	28	36	36
E	S5	S5	5	5	13	13	21	21	29	29	37	37
F	S6	S6	6	6	14	14	22	22	30	30	38	38
G	S7	S7	7	7	15	15	23	23	31	31	39	39
H	S8	S8	8	8	16	16	24	24	32	32	40	40

S1-S8 = Standard Wells
1-40 = 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.

Pre-Wash the Plate

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

Addition of the Reagents

1. **Standards and Samples**

   Add 50 µl of the Mouse IgG1 ELISA Standards or samples into the appropriate wells on the plate.

2. **Anti-Mouse IgG1 ELISA HRP Conjugate**

   Add 50 µl of Anti-Mouse IgG1 ELISA HRP Conjugate (1X) to each well of the plate.

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

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 IgG1 in each sample, making sure to correct for any sample dilution.

Performance Characteristics

Representative Data

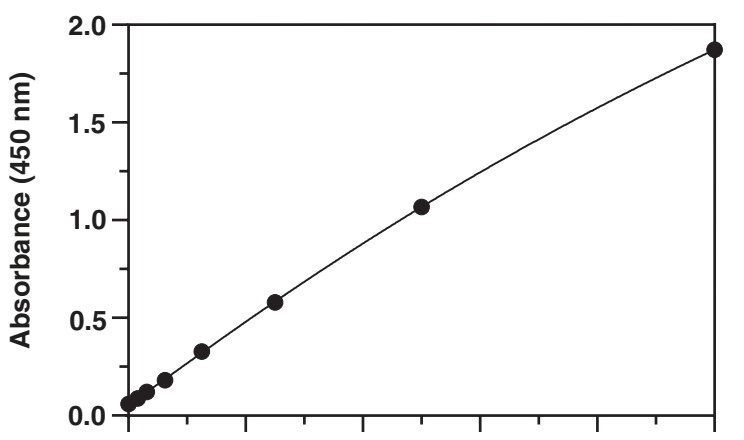
The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve with your experiment. Do not use the data below to determine the values of your samples.

Absorbance at 450 nm

Mouse IgG1 ELISA Standards (ng/ml)	Absorbance at 450 nm	%CV* Intra-Assay Precision	%CV* Inter-Assay Precision
100	1.873	3.1	3.0
50	1.068	2.4	2.3
25	0.580	2.1	2.2
12.5	0.327	2.3	2.0
6.25	0.182	3.1	2.9
3.13	0.121	3.1	4.2
1.56	0.088	6.4	3.8
0	0.059	3.1	16.4

Table 2. Typical results

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



Assay Range = 0-100 ng/ml
Sensitivity (defined as LLOQ) = 1.56 ng/ml
Lower Limit of Detection (LLOD) = 0.23 ng/ml

The sensitivity was derived from the standard curve shown above. The standards were diluted with Immunoassay Buffer D (1X).

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 IgG1 (µg/ml)	%CV
Control 1 (mouse serum)	2,006	5.5
Control 2 (B6 mouse plasma)	554	8.5
Control 3 (BALB/c mouse plasma)	455	6.3

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 IgG1 (µg/ml)	%CV
Control 1 (mouse serum)	2,133	5.0
Control 2 (B6 mouse plasma)	575	9.0
Control 3 (BALB/c mouse plasma)	445	5.3

Table 4. Inter-assay precision

Cross Reactivity:

No cross reactivity was detected with human, rat, rhesus monkey, bovine, sheep, rabbit, hamster, or goat serum diluted 1:10 with Immunoassay Buffer D (1X).

Antibody	Cross Reactivity
Mouse IgG1	100%
Mouse IgG2c	0.025%
Mouse IgE	0.002%
Mouse IgA	0.002%
Mouse IgM	0.002%
Mouse IgG2a	0.002%
Mouse IgG2b	0.002%
Mouse IgG3	0.002%

Table 5. Cross reactivity of the Mouse IgG1 ELISA

RESOURCES

Troubleshooting

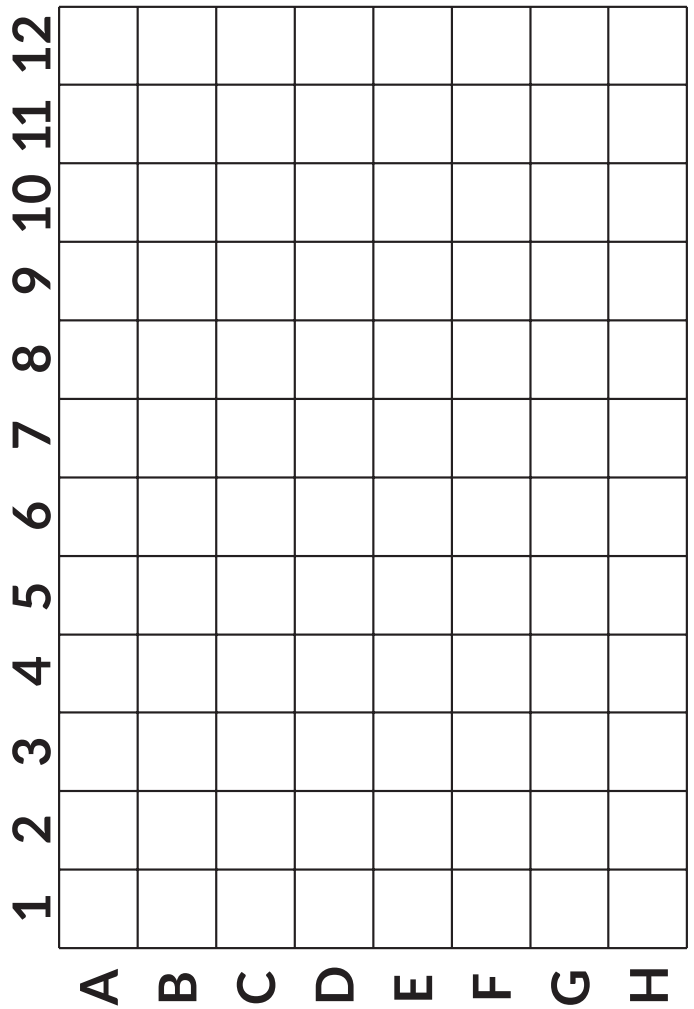
Problem	Possible Causes
Erratic values; dispersion of replicates	A. Trace organic contaminants in the water B. Poor pipetting/technique
Poor development (low signal) of standard curve	A. Trace organic contaminants in the water B. Dilution error in preparing reagents
Poor development (low signal) of samples	A. Sample are too dilute B. Sample is not mouse IgG1-positive
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

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. Lux, A. and Nimmerjahn, F. Of mice and men: The need for humanized mouse models to study human IgG activity in vivo. *J. Clin. Immunol.* **33(Suppl. 1)**, S4-S8 (2013).
3. Vidarsson, G., Dekkers, G., and Rispens, T. IgG subclasses and allotypes: From structure to effector functions. *Front. Immunol.* **5**, 520 (2014).
4. Mayumi, M., Kuritani, T., Kubagawa, H.M., *et al.* IgG subclass expression by human B lymphocytes and plasma cells: B lymphocytes precommitted to IgG subclass can be preferentially induced by polyclonal mitogens with T cell help. *J. Immunol.* **130(2)**, 671-677 (1983).
5. Collins, A.M. IgG subclass co-expression brings harmony to the quartet model of murine IgG function. *Immunol. Cell Biol.* **94(10)**, 949-954 (2016).
6. Martin, R.M., Brady, J.L., and Lew, A.M. The need for IgG2c specific antiserum when isotyping antibodies from C57BL/6 and NOD mice. *J. Immunol. Methods* **212(2)**, 187-192 (1998).

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

Table 6. Assay summary



NOTES

Warranty and Limitation of Remedy

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