Human Therapeutic IgG1 ELISA Kit

Item No. 500910

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Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
400911	Therapeutic IgG1 Assay HRP-Conjugate	1 vial/0.75 ml
400912	Anti-Human IgG1 Precoated 96-Well Strip Plate	1 plate
400914	IgG1 (human) ELISA Standard	1 vial
400108	Immunoassay Buffer D Concentrate (5X)	4 vials/40 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 ea

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 Therapeutic 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 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. An orbital microplate shaker
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. Materials used for **Sample Preparation** (see page 10)
5. A source of pure water; glass-distilled or HPLC-grade water is acceptable.

INTRODUCTION

Background

Human therapeutic antibodies have become increasingly common components of early drug discovery and development portfolios in the pharmaceutical and biotech industries.[1] As part of the preclinical toxicology assessment of these agents, they are routinely tested in non-human primates, primarily in rhesus or cynomolgus monkeys.[2-4] In order to assess the pharmacokinetics of the human antibodies in monkey serum, it is necessary for an assay to be capable of distinguishing the experimentally introduced human IgG from the endogenous monkey IgG.[2,4] Historically, this has proven difficult due to the high degree of homology between these immunoglobulin species.[2] Cayman's Human Therapeutic IgG1 ELISA Kit accurately measures human IgG in monkey serum for use in the pharmacokinetic analysis of therapeutic human antibodies.

Most clinically approved therapeutic human antibodies are of the IgG1, IgG2, and IgG4 isotypes.[1,3,5] IgG1 is commonly used because of its effector functions: it binds with high affinity to Fc receptors on effector leukocytes and fixes complement. Thus, IgG1 mediates antibody-induced cellular cytotoxicity (ADCC) as well as complement-mediated cellular cytotoxicity.[5,6] For some therapeutic applications, neither ADCC nor complement activation is desired, in which case the IgG2 or IgG4 isotypes are often employed. Cayman offers ELISA kits optimized specifically for the detection of human IgG1, human IgG2, or human IgG4.

About This Assay

Cayman's Human Therapeutic IgG1 ELISA Kit is an immunometric (*i.e.* sandwich) assay that can be used for the quantification of human IgG1 in monkey, rat, mouse, or rabbit plasma and serum without prior sample purification. The standard curve spans the range of 3.13-200 ng/ml, with a lower limit of quantification (LLOQ) of 6.25 ng/ml. The assay has been validated in serum from cynomolgus monkey, rhesus monkey, rat, mouse, and rabbit.

Although a human monoclonal IgG1 antibody is supplied as a standard, we acknowledge that minor differences in protein structure or post-translational modification may exist between our standard and the human IgG1 test article that is being assessed by the customer, and that these differences could lead to inaccuracies in the quantification of the test article. Thus, we anticipate that many of our customers will prefer to use their own purified therapeutic monoclonal IgG1 as the standard in this ELISA. In that event, we recommend that the customer test their own standard in the ELISA at a similar dose range as the standard provided in the kit.

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 an antibody specific for human IgG. This antibody will bind any human IgG introduced into the well. A second antibody conjugated to horseradish peroxidase (HRP), which also recognizes human IgG, 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 IgG 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 IgG1.

$$\text{Absorbance} \propto [\text{Anti-human IgG HRP}] \propto [\text{IgG1}]$$

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

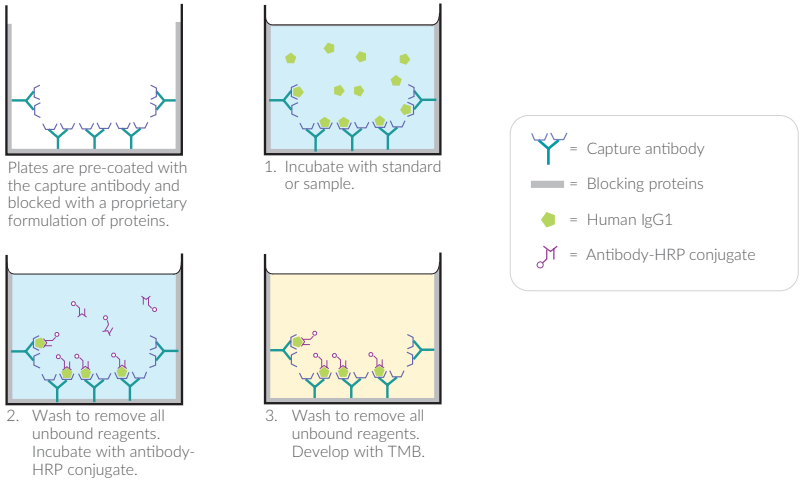


Figure 1. Schematic of the ELISA

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 mean absorbance (450 nm) – (1.645 x S.D.) is higher than the mean zero value absorbance (450 nm) + (1.645 x S.D.).

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 pure 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 pure 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 pure water to a total volume of 2 L and add 1 ml of Polysorbate 20.

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, monkey, rat, mouse, or rabbit serum or plasma (prepared using heparin or EDTA as the anticoagulant) can be used directly in the assay following dilution in Assay Buffer (1X).

Preparation of Assay-Specific Reagents

IgG1 (human) ELISA Standard

Reconstitute the lyophilized IgG1 (human) ELISA Standard (Item No. 400914) with 1.5 ml of Assay Buffer (1X) and mix gently. The concentration of this solution (the bulk standard) will be 200 ng/ml. The reconstituted standard will be stable for approximately two weeks when stored at 4°C.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 250 µl of Assay Buffer (1X) to tubes #2-8. Transfer 500 µl of the bulk standard (200 ng/ml) to tube #1. Serially dilute the standard by removing 250 µl from tube #1 and placing it in tube #2; mix gently. Next, remove 250 µl from tube #2 and place it into tube #3; mix gently. Repeat this process for tubes #4-7. Do not add any IgG1 to tube #8. This tube is the zero-point vial, the lowest point on the standard curve. These diluted standards should not be stored for more than four hours.

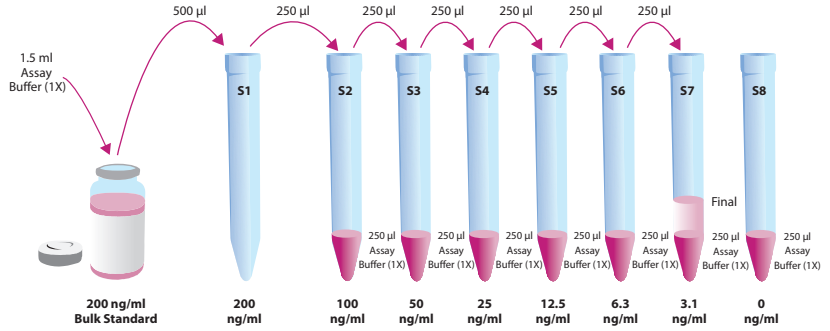


Figure 2. Preparation of the IgG1 standards

Therapeutic IgG1 Assay HRP-Conjugate

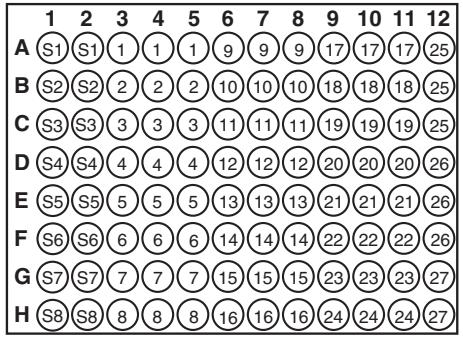
The Therapeutic IgG1 Assay HRP Conjugate (Item No. 400911) is supplied as a concentrated (20X) stock solution of goat anti-human antibody conjugated to HRP. Immediately before addition to the plate, prepare a 1X working solution by adding 0.6 ml of the concentrated conjugate to 11.4 ml Assay Buffer (1X) for a full plate (12 ml total) or 0.3 ml of the concentrated conjugate to 5.7 ml of Assay Buffer (1X) for a half plate (6 ml total).

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 according to the plate insert 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, on page 13. 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 26).



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. Pipette 100 µl of the IgG1 (human) ELISA Standards or samples into 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) and incubate for two hours at room temperature on an orbital shaker.

Addition of the Therapeutic IgG1 Assay HRP-Conjugate Working Solution and Second Incubation

1. Empty the wells and rinse four 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 Therapeutic IgG1 Assay HRP-Conjugate as described in the **Preparation of Assay-Specific Reagents** section (see page 11).
3. Add 100 µl of the Therapeutic IgG1 Assay HRP-Conjugate working solution to each well of the plate.
4. Cover the plate with the 96-Well Cover Sheet 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 ~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 10 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.

Calculations

Plot the Standard Curve and Determine the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) *versus* concentration (log x-axis) for standards (S1-S8) and fit the data with a four-parameter logistic equation. Using the equation of the line, calculate the concentration of analyte 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. Your results could differ substantially. Development of the plate for 10 minutes typically results in an absorbance of >1.2 optical density (O.D.) units for the 200 ng/ml standard.

IgG1 Standards (ng/ml)	Absorbance	%CV* Intra-Assay Precision (tested in 4% cynomolgus monkey serum)	%CV* Inter-Assay Precision (tested in 10% cynomolgus monkey serum)
200	1.756	5.4	0.01
100	1.151	3.5	0.2
50	0.649	7.0	0.7
25	0.347	5.7	1.1
12.5	0.196	15.9	1.6
6.3	0.126	13.6	2.8
3.1	0.090	19.6	17.6

Table 1. Typical results
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve

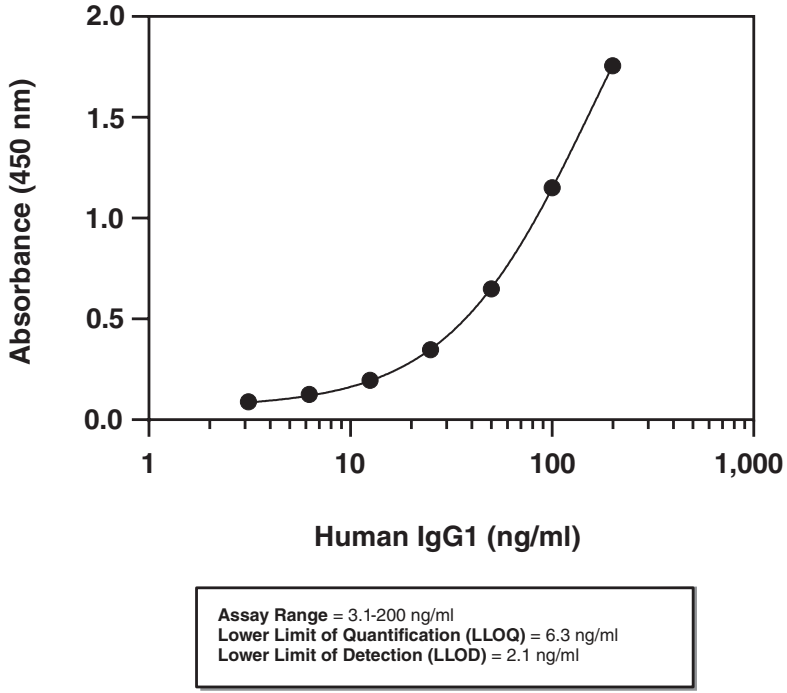


Figure 4. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 15 replicates of two cynomolgus monkey serum controls in a single assay.

Matrix Control (ng/ml)	%CV
48.2*	4.6
58.0**	3.9

Table 2. Intra-assay precision
*In 5% cynomolgus monkey serum
**In 10% cynomolgus monkey serum

Inter-assay precision was determined by analyzing replicates of two cynomolgus monkey serum controls in three separate assays on two different days.

Matrix Control (ng/ml)	%CV
47.7*	2.7
53.3**	3.0

Table 3. Inter-assay precision
*In 5% cynomolgus monkey serum
**In 10% cynomolgus monkey serum

IgG1 (ng/ml)	Mean of O.D.	Standard Deviation (S.D.)	O.D. - (1.645 x S.D.)
200	2.284	0.08	2.158
100	1.341	0.04	1.274
50	0.691	0.05	0.613
25	0.363	0.02	0.332
12.5	0.206	0.03	0.163
6.3	0.126	0.01	0.111
3.1	0.091	0.004	0.084
0	0.074	0.006	0.084*

*O.D. + (1.645 x S.D.)

Table 4. Determination of LLOQ

The LLOQ is defined as the lowest standard concentration in which mean absorbance - (1.645 x S.D.) is higher than the blank value mean absorbance + (1.645 x S.D.). The LLOQ is 6.3 ng/ml.

Matrix Validation:

The Therapeutic Human IgG1 ELISA Kit, including the standard, does not contain serum of any species. The IgG1 standards have been analyzed in this assay in the presence of 1%, 5%, and 10% cynomolgus monkey, rat, mouse, and rabbit sera with no significant change in the characteristics of the assay (see Figure 5). When analyzing samples containing greater than 5% cynomolgus monkey serum, it is recommended to dilute the standards in an equivalent serum concentration for precise IgG1 measurements.

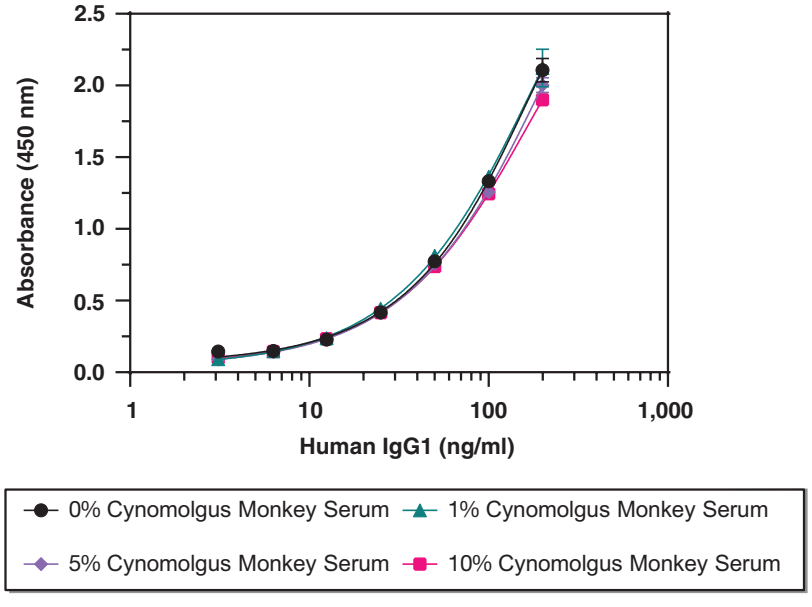


Figure 5. Comparison of human IgG1 ELISA results in different concentrations of cynomologus monkey serum

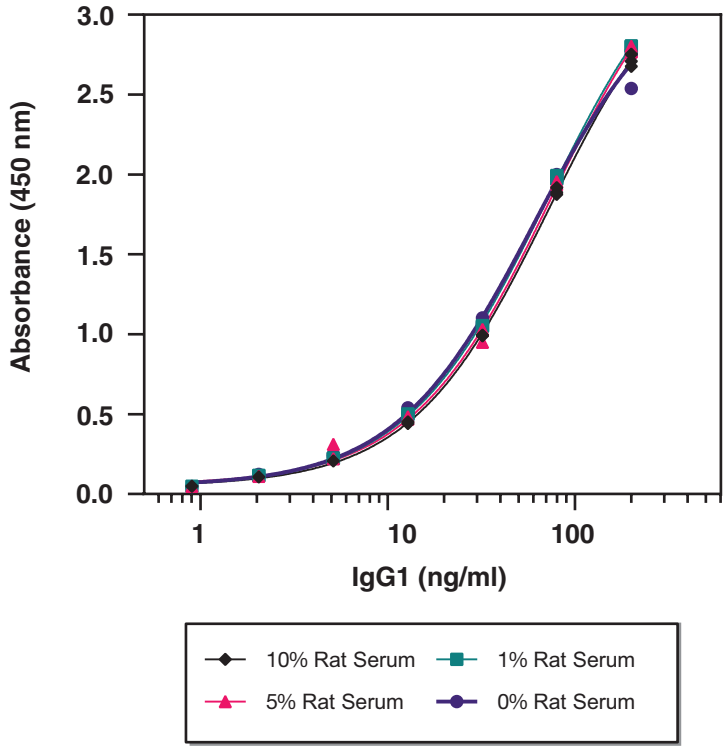


Figure 6. Comparison of human IgG1 ELISA results in different concentrations of rat serum

NOTE: Data in mouse and rabbit sera is available upon request. Please contact Cayman's Technical Service team at techserv@caymanchem.com or 888-526-5351.

Spike and Recovery:
Cynomolgus monkey serum was spiked with different amounts of human IgG1, serially diluted with Assay Buffer (1X), and evaluated using the Therapeutic Human IgG1 ELISA Kit. The error bars represent standard deviations obtained from multiple dilutions of each sample.

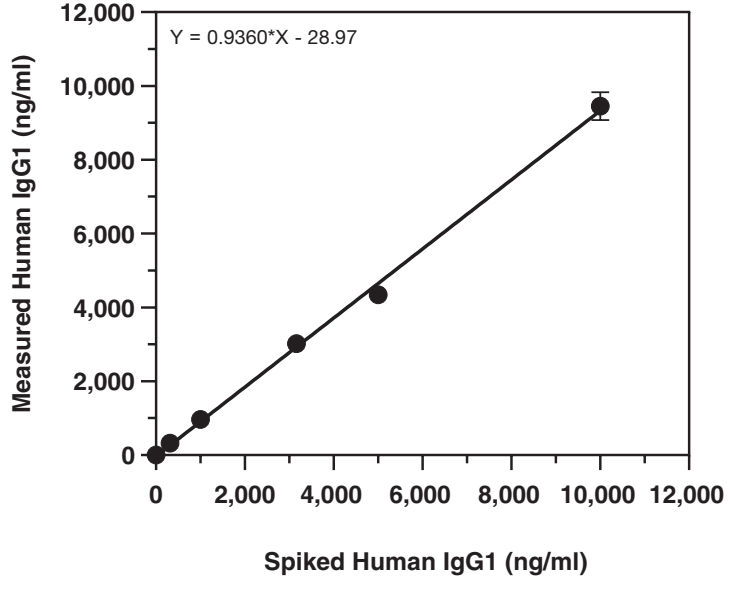


Figure 7. Spike and recovery of human IgG1 in cynomolgus monkey serum

Linearity:
Cynomolgus monkey serum was spiked with 4,000 ng/ml of human IgG1, serially diluted with Assay Buffer (1X), and evaluated for linearity using the Human Therapeutic IgG1 ELISA Kit. The results are shown in Table 5 below.

Dilution	Measured Concentration (ng/ml)	Linearity (%)
Cynomologus Monkey Serum		
20	3,692.6	100%
40	3,716.5	101%
80	3,891.4	105%
160	3,786.9	103%

Table 5. Linearity in cynomolgus monkey serum

RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
Poor development (low signal) of standard curve	A. Trace organic contaminants in the water source B. Dilution error in preparing reagents

References

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2. Stubenrauch, K., Wessels, U., and Lenz, H. *J. Pharm. Biomed. Anal.* **49(4)**, 1003-8 (2009).
3. Chan, A.C. and Carter, P.J. *Nat. Rev. Immunol.* **10(5)**, 301-16 (2010).
4. Yang, J., Ng, C., Lowman, H., *et al. J. Immunol. Methods* **335(1-2)**, 8-20 (2008).
5. Wang, W., Wang, E.Q., and Balthasar, J.P. *Nature* **84(5)**, 548-558 (2008).
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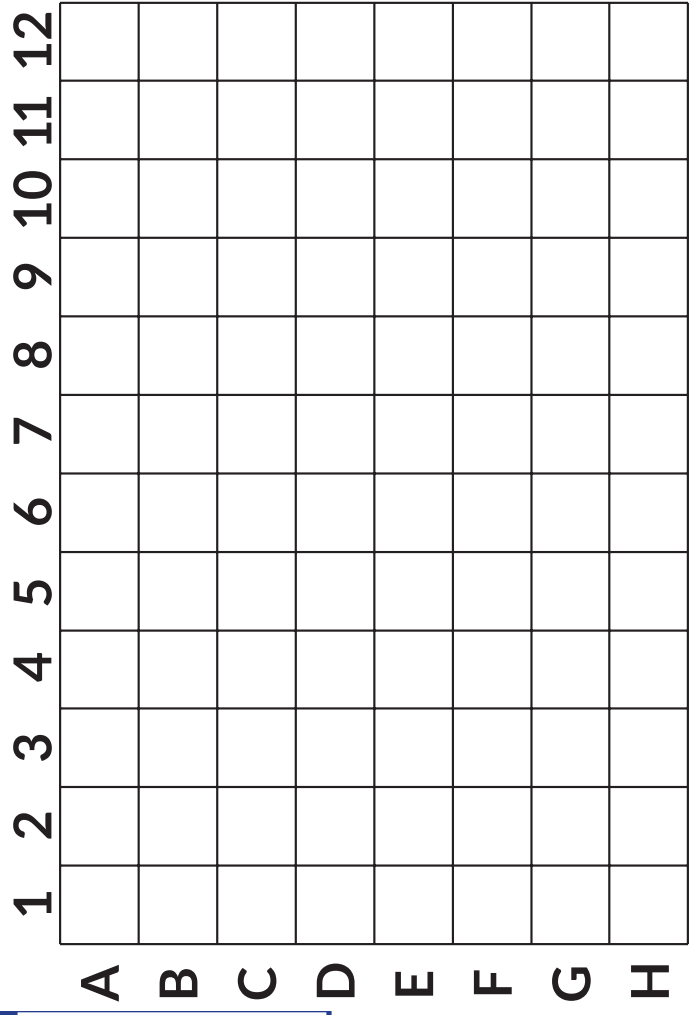

NOTES

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.