Human Therapeutic IgG2 ELISA Kit

Item No. 500960

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	96 wells Quantity/Size
400961	Therapeutic IgG2 Assay HRP-Conjugate	1 vial/1.5 ml
400962	Anti-Human IgG2 Precoated 96-Well Strip Plate	1 plate
400964	IgG2 (human) ELISA Standard	1 vial
400054	Immunoassay Buffer B 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 Human Therapeutic IgG2 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

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

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 with the ability to measure absorbance at 450 nm.
2. Adjustable pipettes and a repeating pipettor.
3. Materials used for **Sample Preparation** (see page 11).
4. A source of pure water; glass distilled water 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. As part of preclinical toxicology assessment of these agents, they are routinely tested in non-human primates, primarily in rhesus or cynomolgus monkeys. In order to assess the pharmacokinetics of the human antibodies in monkey serum, it is necessary to have an assay that is capable of distinguishing the experimentally-introduced human IgG from the endogenous monkey IgG.[1,2] Historically, this has proven difficult due to the high degree of homology between these immunoglobulin species.[1,2] Cayman has developed an ELISA format that accurately measures human IgG in monkey serum for use in the pharmacokinetic analysis of therapeutic human antibodies.

The vast majority of clinically-approved therapeutic human antibodies are of the IgG1, IgG2, and IgG4 isotypes.[3] IgG1 is most commonly used because of its "effector" functions: 1) it binds with high affinity to Fc receptors on effector leukocytes, and 2) it fixes complement. Thus, IgG1 mediates antibody-induced cellular cytoxicity (ADCC) as well as complement-mediated cellular cytoxicity.[4,5] For some therapeutic applications, neither ADCC nor complement activation is desired, in which case the IgG2 or IgG4 isotypes are usually employed.[4,5] Cayman offers ELISA kits optimized specifically for the detection of human IgG1, human IgG2, or human IgG4.

About This Assay

Although a human monoclonal IgG2 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 IgG2 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 IgG2 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.

Cayman's Human Therapeutic IgG2 ELISA Kit is an immunometric assay which can be used to measure human IgG2 in monkey or rodent 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 3.13 ng/ml. The assay has been validated in sera from rats and cynomolgus monkeys.

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 human IgG. This antibody will bind any human IgG introduced into the well. A second antibody recognizing a different epitope of human IgG (Antibody/HRP Conjugate) is added to the well. This allows the two antibodies to form a 'sandwich' by binding on opposite sides of the human 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 human 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.

$$\text{Absorbance} \propto [\text{Anti-human IgG/HRP}] \propto [\text{IgG2}]$$

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

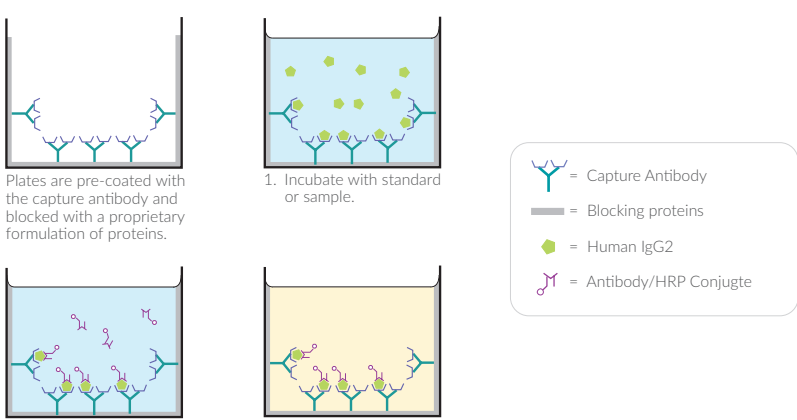


Figure 1. Schematic of the Immunometric ELISA

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.

PRE-ASSAY PREPARATION

Buffer Preparation

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

1. **Assay Buffer Preparation**

   Dilute the contents of one vial of Immunoassay Buffer B Concentrate (10X) (Item No. 400054) with 90 ml of distilled 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 distilled water.*

2. **Wash Buffer Preparation**

   5 ml vial Wash Buffer Concentrate (400X) (Item No. 400062): Dilute to a total volume of 2 L with distilled 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 general, monkey or rodent serum or plasma (prepared using heparin or EDTA as the anticoagulant) can be used directly in the assay following dilution in Assay Buffer.

Preparation of Assay-Specific Reagents

IgG2 (human) ELISA Standard

Reconstitute the lyophilized purified IgG2 (human) ELISA Standard (Item No. 400964) with 1.5 ml of Assay Buffer. Mix gently. The concentration of this solution (the bulk standard) is 200 ng/ml. The reconstituted standard should be stable for two weeks at 4°C.

To prepare the standard for use in the ELISA: Obtain eight clean test tubes and label them, #1 through #8. Aliquot 250 µl of Assay Buffer into tubes #2-8. Transfer 500 µl of freshly prepared stock standard (200 ng/ml) to tube #1. Serially dilute the standard by removing 250 µl from tube #1 and placing into tube #2. Mix gently. Next, remove 250 µl from tube #2 and place into tube #3; mix gently. Repeat this process for tubes #4-7. Do not add any IgG2 to tube #8. This tube is the zero-point vial, the lowest point on the standard curve.

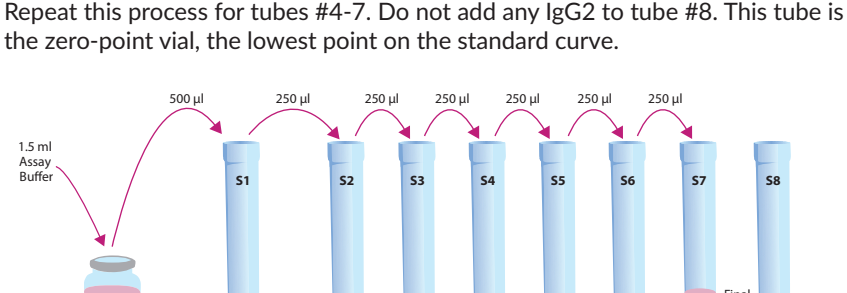


Figure 2. Preparation of the IgG2 standards

Therapeutic IgG2 Assay HRP-Conjugate

This reagent is supplied as a concentrated (20X) stock solution of sheep anti-human IgG polyclonal antibody conjugated to HRP. On the day of the assay, prepare a 1X working solution by adding 0.6 ml of the Therapeutic IgG2 Assay HRP-Conjugate (Item No. 400961) to 11.4 ml Assay Buffer (12 ml total). ***Use HRP-Conjugate diluted in Assay Buffer within four hours.*** Sufficient concentrated stock solution has been provided to produce an additional 12 ml of the 1X 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 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, 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 17, for more details). We suggest you record the contents of each well on the template sheet provided (see page 27).

	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 - Standards 1-8
1-27 - Samples

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 two hours 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 10 minutes at room temperature in the dark 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. *NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/immuno) to obtain a free copy of this convenient data analysis tool.*

Calculations

Plotting the Standard Curve and Determining the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) *versus* concentration (log x-axis) for standards (S1-S7) and fit the data with a four-parameter logistic equation, or alternatively a smoothed cubic spline.

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.

Human IgG2 (ng/ml)	Absorbance	
200	1.210	1.245
100	1.055	1.087
50	0.848	0.851
25	0.56	0.565
12.5	0.319	0.326
6.25	0.179	0.162
3.13	0.087	0.087
0	0.024	0.002

Table 1. Typical results

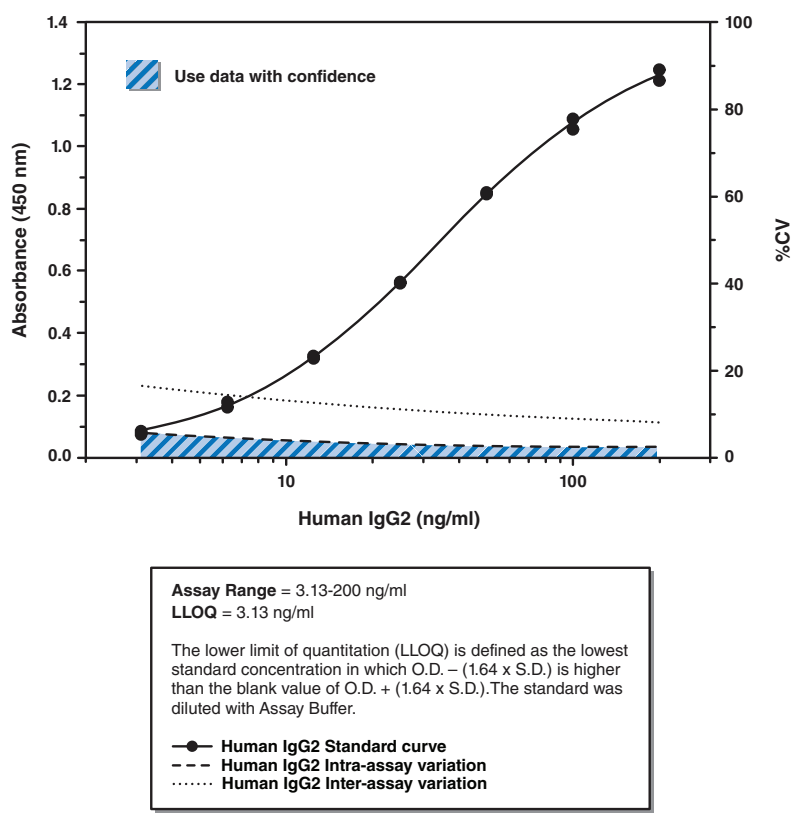


Figure 4. Typical standard curve

Precision:

The intra- and inter-assay CVs have been determined at multiple points on the standard curve. These data are summarized in the graph on page 19 and in the table below.

IgG2 (ng/ml)	%CV* Intra-assay variation	%CV* Intra-assay variation in 5% cynomolgus monkey serum	%CV* Inter-assay variation
200	19.27	17.45	9.79
100	9.19	11.09	7.23
50	4.17	5.10	6.79
25	5.83	6.63	13.89
12.5	4.60	4.72	14.21
6.25	5.49	7.37	13.52
3.13	6.00	6.09	15.97
0	†	†	†

Table 2. Intra- and inter-assay variation
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.
†Outside of the recommended usable range of the assay.

IgG2 (ng/ml)	Mean O.D.	Standard Deviation (S.D.)	O.D. - (1.64 x S.D.)
200	1.247	0.034	1.247
100	1.042	0.046	0.966
50	0.798	0.056	0.706
25	0.548	0.016	0.522
12.5	0.331	0.014	0.308
6.25	0.198	0.020	0.164
3.13	0.126	0.006	0.116
0	0.075	0.008	0.087*

*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 3.13 ng/ml.

Matrix Validation:

The ELISA Kit and standards do not contain serum of any species. The IgG2 standards have been analyzed in this assay in the presence of 1%, 5%, and 10% cynomolgus monkey and rat serum with no significant change in the characteristics of the assay (see Figures 5 and 6). When analyzing samples containing greater than 5% cynomolgus serum, we recommend diluting the standards in an equivalent cynomolgus monkey serum concentration.

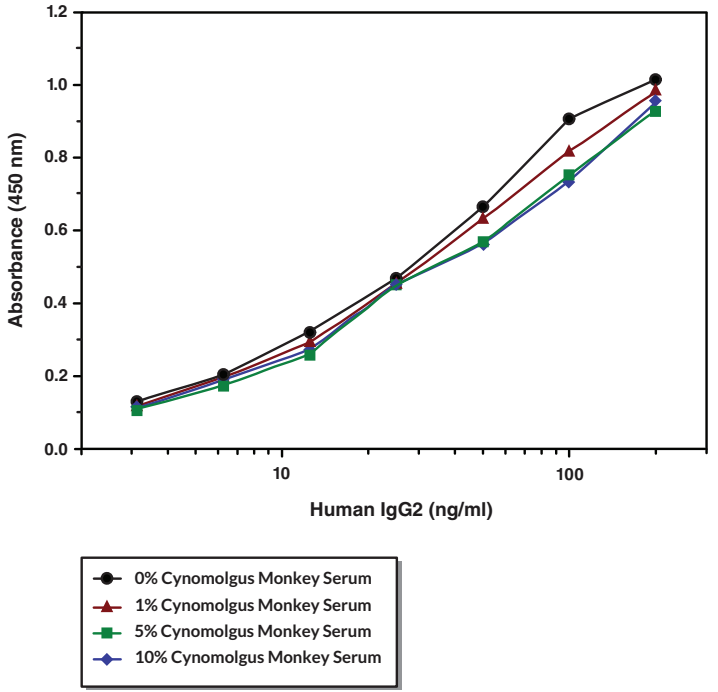


Figure 5. Comparison of human IgG2 ELISA results in different concentrations of cynomolgus monkey serum

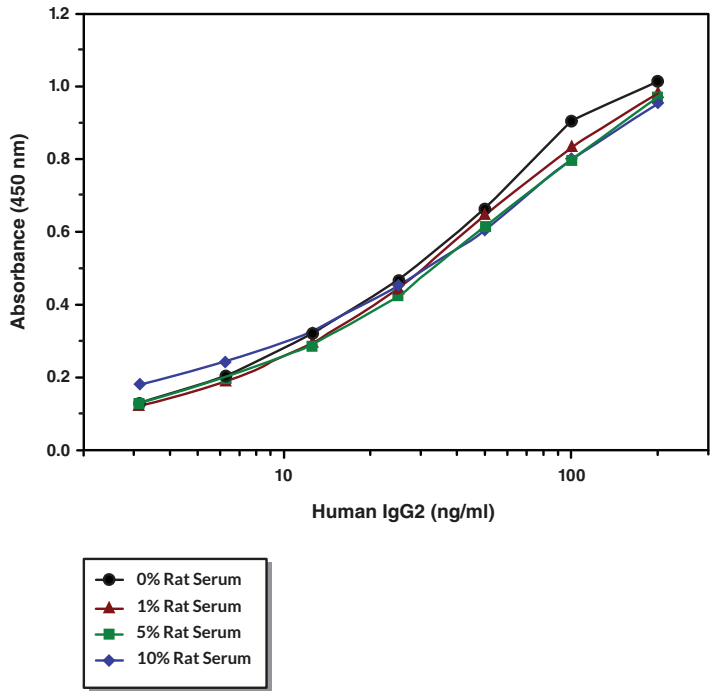


Figure 6. Comparison of human IgG2 ELISA results in different concentrations of rat serum

Spike Recovery:
Human IgG2 was spiked into 100% cynomolgus serum at a concentration of 4.0 µg/ml and then diluted with Immunoassay Buffer B containing 5% cynomolgus monkey serum to achieve the target concentrations.

Target (ng/ml)	Assayed Result (ng/ml)	% Recovery
200	186.16	93.08
100	103.00	103.00
50	54.50	109.00
25	28.05	114.00
12.5	13.66	109.28
6.25	6.32	101.12
3.13	3.40	108.80

Table 4. Recovery of human IgG from cynomolgus monkey serum

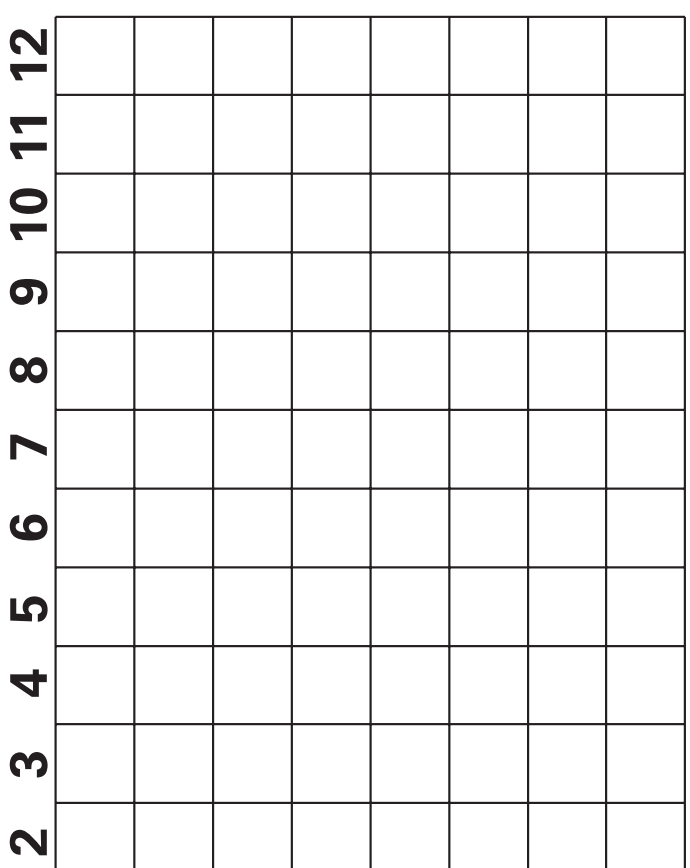
RESOURCES

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	A. Repeat assay with longer development time B. Perform assay again using correct standard dilutions C. Obtain new standard

References

1. Yang, J., Ng, C., Lowman, H., *et al.* Quantitative determination of humanized monoclonal antibody rrhuMAb2H7 in cynomolgus monkey serum using a generic immunoglobulin pharmacokinetic (GRIP) assay. *J. Immunol. Methods* **335(1-2)**, 8-20 (2008).
2. Stubenrauch, K., Wessels, U., and Lenz, H. Evaluation of an immunoassay for human-specific quantitation of therapeutic antibodies in serum samples from non-human primates. *J. Pharm. Biomed. Anal.* **49(4)**, 1003-8 (2009).
3. Chan, A.C. and Carter, P.J. Therapeutic antibodies for autoimmunity and inflammation. *Nat. Rev. Immunol.* **10**, 301-16 (2010).
4. van Dijk, M.A. and van de Winkel, J.G. Human antibodies are next generation therapeutics. *Curr. Opin. Chem. Biol.* **5(4)**, 368-74 (2001).
5. Salfeld, J.G. Isotype selection in antibody engineering. *Nat. Biotech.* **25**, 1369-1372 (2007).



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.