Human IgG4 ELISA Kit

Catalog Number：RK00376

This ELISA kit used for quantitation of Human IgG4 concentration in cell culture supernate, serum and plasma. For research use only, and it's highly recommended to read throughly of this manual before using the product.

Manufactured by

Global Headquarters
500W Cummings Park
Ste. 6500 Woburn, MA 01801

Tel :888-754-5670
E-mail:info@abclonal.com
http: www.abclonal.com

China Branch
388# Gaoxin Road (No.2)
East Lake Development Zone
Wuhan P. R. China

Tel: 400-999-6126
E-mail: market@abclonal.com
http: www.abclonal.com.cn

Order: order@abclonal.com
Tech: support@abclonal.com

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Introduction

Immunoglobulin G (IgG) is synthesized and secreted by B cells and accounts for immunoglobulin in serum at 75%, it is the type of antibody with the highest proportion in the peripheral circulation.IgG has four subtypes,they are IgG1, IgG2, IgG3 and IgG4, respectively.IgG4 is the lowest level of SERUM IgG

Isoforms, accounting for 4% of the total IgG, have an estimated concentration of 0.35 -- 0.51 mg/ml in the body,but it varies from person to person, and the concentration of IgG4 in healthy people can range from 10ug/ml to 1.4mg /ml.

The IgG4 heavy chain was more than 95% homologous to the other three IgG subtypes.Many cytokines are involved and promote the production of IgG4, and these cytokines and promote the production of IgE cell children are similar, such as IL-4,IL-5,IL-6,IL-7,IL-9,IL-13. In addition, IL-10 and IL-12 are also involved

Promote IgG4 production.A series of inflammatory diseases caused by elevated IgG4 levels in the body are known as Igg4-related diseases include salivary adenitis, pancreatitis, cholangitis, aortitis, and nail adenitis, etc.

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Principle Of The Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for IgG4 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IgG4 present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for IgG4 is added to the wells and binds to the combination of capture antibody-IgG4 in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps, a substrate is added. A colored product TMB is formed in proportion to the amount of IgG4 present in the sample. The reaction is terminated by addition of acid and absorbance is measured. A standard curve is prepared from seven IgG4 standard dilutions and IgG4 sample concentration determined.

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Materials Provided

Part	Size (96T)	Cat NO.	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Antibody Coated Plate	**8×12**	**RM01532**	Return unused wells to the foil pouch containing the desiccant pack and store at ≤ -20 °C.Reseal along entire edge of zip-seal.
Standard Lyophilized	**3**	**RM01529**	Aliquot and store at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Concentrated HRP-Conjugate antibody (30×)	**1 ×400ul**	**RM01530**	May be stored for up to 6 month at 2-8 °C.*
Standard/Sample Diluent (R1)	**1 ×20mL**	**RM00023**	May be stored for up to 6 month at 2-8 °C.*
HRP-Conjugate antibody Diluent (R2)	**1 ×16mL**	**RM00024**	
Wash Buffer(20x)	**1 × 30mL**	**RM00026**	
TMB Substrate	**1 ×12 mL**	**RM00027**	
Stop Solution	**1 ×12 mL**	**RM00028**	
Plate Sealers	**4 strips**		
Specification	**1**		

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Sample Collection And Storage

1. **Cell Culture Supernates:**

   Centrifuge 1000x g for 10 min and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.If cell culture supernate samples require larger dilutions, perform an intermediate dilution with culture media and the final dilution with the Standard/Sample Diluent(R1).

2. **Serum:**

   Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000x g, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.

3. **Plasma**

   Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze / thaw cycles.

4. **Avoid hemolytic and hyperlipidemia sample for Serum and Plasma.**

5. **Dilution:**

   Dilute samples at the appropriate multiple (recommend to do pre-test to determine the dilution factor).

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Precautions

1. **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
2. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
3. Variations in sample collection, processing, and storage may cause sample value differences.
4. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
5. Stop Solution contains strong acid. Wear eye, hand, and face protection.
6. Apart from the standard of kits, other components should not be refrigerated.
7. Please perform simple centrifugation to collect the liquid before use.
8. Do not mix or substitute reagents with those from other lots or other sources.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
10. Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all of the diluent.
11. Both the sample and standard should be assayed in duplicate, and the sequence of the regents should be added consistently.
12. Reuse of dissolved standard is not recommended.
13. The kit should not be used beyond the expiration date on the kit label.
14. The kit should be away from light when it is stored or incubated.

15. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
16. To avoid cross contamination, please use disposable pipette tips.
17. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
18. The 48T kit is also suitable for the specification.

Experiment Materials

1. Microplate reader(measuring absorbance at 450 nm, with the correction wavelength set at 570 nm or 630 nm).
2. Pipettes and pipette tips：0.5-10, 2-20, 20-200, 200-1000 μL.
3. Microplate washer, Squirt bottle.
4. Micro-oscillator.
5. Deionized or double distilled water, graduated cylinder.
6. Polypropylene Test tubes for dilution.
7. Incubator.

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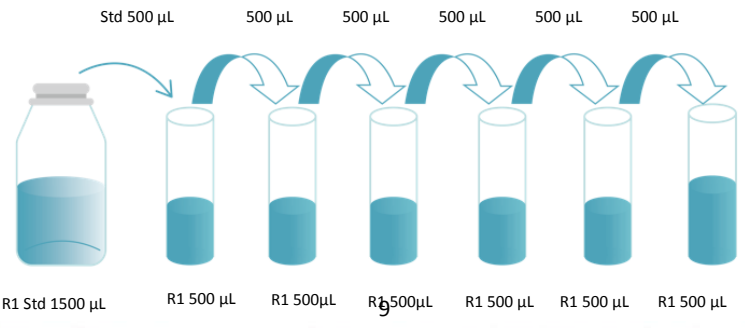
Reagent Preparation

1. **Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.**

2. **Standard:** Add Standard/Sample Diluent(R1) 1.5mL into freeze-dried standard, sit for a minimum of 15 minutes with gentle agitation prior to making dilutions (1000ng/mL), Prepare EP tubes containing Standard/Sample Diluent(R1), and produce a dilution series according to the picture shown below (recommended concentration for standard curve: 1000, 500, 250, 125, 62.5,31.2, 15.6, 0ng/mL). Redissolved standard solution (1000ng/mL), aliquot and store at -20°C— -70°C.



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3. **Concentrated HRP-Conjugate antibody (30x)** : Dilute 1:30 with the HRP-Conjugate antibody Diluent (R2) before use, and the diluted solution should be used within 30 min.

Dilution Method

Strip	Concentrated HRP-Conjugate antibody (30x)	HRP-Conjugate antibody Diluent (R2)
2	66uL	1914uL
4	132uL	3828uL
6	198uL	5742uL
8	264uL	7656uL
10	330uL	9570uL
12	396uL	11484uL

4. **Wash buffer**: Dilute 1:20 with double distilled or deionized water before use.

Wash Method

Aspirate each well and wash, repeating the process two times for a total of three

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washes.Wash by filling each well with **Wash Buffer**(300ul) using a squirt bottle,manifold dispenser,or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash,remove any remaining **Wash Buffer** by aspirating or decanting.Invert the plate and blot it against clean paper towels.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Add 100 µL Standard/sample Diluent (R1) in blank well.
3. Add 100 µL different concentration of standard and sample in other wells, cover with the adhesive strip provided. Incubate for 2 hours at 37°C.
4. Add wash buffer 300 µL/well, aspirate each well after holding 40 seconds, repeating the process four times for a total of five washes.
5. Prepare the Concentrated HRP-Conjugate antibody (30X) Working Solution 15 minutes early before use.
6. Add HRP-Conjugate antibody Diluent(R2) in blank well and Biotin-Conjugate antibody Working Solution in other wells (100µL/well), cover with new adhesive strip provided. Incubate for 1 hour at 37°C.
7. Warm-up the Microplate reader.
8. Repeat the aspiration/wash as in step 4.
9. Add TMB Substrate (100µL/well). Incubate for 15-20 minutes at 37°C .Protect from light.
10. Add Stop Solution (100µL/well), determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is

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not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

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Assay Procedure Summary

Prepare the standard and regents

↓

Add 100ul of standards and test samples to each well

Incubate for 2 hours at 37°C ↓ ***wash 5 times***

Add 100ul HRP-Conjugate antibody Working Solution

Incubate for 1 hour at 37°C ↓ ***wash 5 times***

Add 100ul Substrate Solution

Incubate for 15-20 min ↓ ***at 37°C under dark condition.***

Add 100ul Stop Solution

↓

Detect the optical density within 5 minutes under 450nm.

Correction Wavelength set at 570nm or 630nm

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Calculation Of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).
2. Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the IgG4 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted,the concentration read from the standard curve must be multiplied by the dilution factor.

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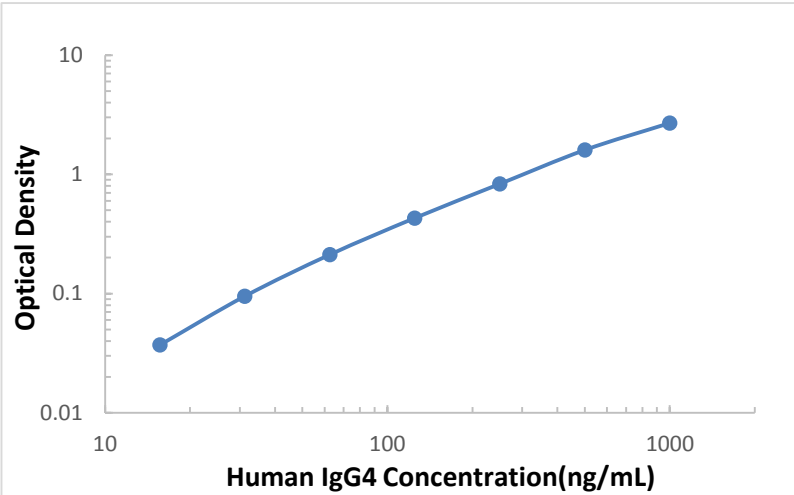
Typical Data



The standard curves are provided for demonstration only. A standard curve should be generated for each set of IgG4 assayed.

Sensitivity

The minimum detectable dose (MDD) of IgG4 is typically less than 7.8ng/mL. The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

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Specificity

This assay recognizes both recombinant and natural mouse IgG4. The factors listed below were prepared at 50ng/ml and assayed for cross-reactivity. No significant cross-reactivity was observed with the following:

Recombinant human

IgG1

IgG2

IgG3

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Precision

Intra-plate Precision

Three samples of known concentration were tested 20 times on one plate to evaluate the Intra-plate precision.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	175	580	890
Standard Deviation (SD)	5.4	16.2	32.9
Variable Coefficient CV (%)	3.1	2.8	3.7

Inter-plate Precision

Three samples of known concentration were tested 20 times separate assays to evaluate the Inter-plate precision.Assay were using two lots of components.

Sample	1	2	3
Repeat Times	20	20	20
Average Value (pg/mL)	80	410	820
Standard Deviation (SD)	5.0	29.1	60.6
Variable Coefficient CV (%)	6.2	7.1	7.4

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Recovery

Spike 3 different concentration of mouse IgG4 into healthy mouse serum and plasma, calculate the recovery.

Sample Form	Average Recover (%)	Range (%)
Serum	92	87-96
Plasma	91	83-99

Linearity

Spike high concentration of mouse IgG4 into 4 healthy mouse serum, dilute in the range of standard curve kinetics and evaluate the linearity.

Dilution	Average Value (%)	Range (%)
1:2	94	86-102
1:4	94	88-99
1:8	88	85-90
1:16	98	90-105

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