Rabbit IgG ELISA kit

Enzyme Immunoassay for the quantification of Rabbit IgG in Rabbit Serum, plasma and cell culture supernatants.

Catalog number: ARG82958

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION **Page**

INTRODUCTION 3

PRINCIPLE OF THE ASSAY 3

MATERIALS PROVIDED & STORAGE INFORMATION 4

MATERIALS REQUIRED BUT NOT PROVIDED 4

TECHNICAL HINTS AND PRECAUTIONS 5

SAMPLE COLLECTION & STORAGE INFORMATION 6

REAGENT PREPARATION 7

ASSAY PROCEDURE 9

CALCULATION OF RESULTS 11

EXAMPLE OF TYPICAL STANDARD CURVE 12

QUALITY ASSURANCE 12

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Tel: +886-3-6221320

Fax: +886-3-5530266

Email: info@arigobio.com

INTRODUCTION

Immunoglobulin G (IgG) is a type of antibody. Representing approximately 75% of serum antibodies in humans, IgG is the most common type of antibody found in blood circulation. IgG molecules are created and released by plasma B cells. Each IgG antibody has two paratopes.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for IgG has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any IgG present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for IgG is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of IgG bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of IgG in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Expiration date: Six months at 4°C and twelve months at-20°C

NO	Component	Quantity	Storage information
C1	Antibody-coated microplate	8 X 12 strips	4°C
C2	Standard (Lyophilized)	3 X 5 ng/Vial	4°C
C3	Standard diluent	20 ml (Ready to use)	4°C
C4	Antibody conjugate concentrate	1 vial (400 µl)	4°C
C5	Antibody diluent buffer	16 ml (Ready to use)	4°C
C6	HRP-Streptavidin concentrate	1 vial (400 µl)	4°C
C7	HRP-Streptavidin diluent buffer	16 ml (Ready to use)	4°C
C8	20X Wash buffer	50 ml	4°C
C9	TMB substrate	12 ml (Ready to use)	4°C (Protect from light)
C10	STOP solution	12 ml (Ready to use)	4°C
C11	Plate sealer	6 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times. Reconstituted standard should be aliquoted and stored at -20°C or -80°C (-80°C is recommended) to avoid repeated freeze-thaw cycles.
- Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8 °C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C for 15 min or until the crystals are completely dissolved.
- To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
- The TMB Color developing agent should be colorless and transparent before using.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Do not let strips dry, as this will inactivate active components in wells.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

- Avoid using reagents from different batches.
- In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the 1X HRP-Streptavidin Solution and TMB substrate be pre-warmed in 37°C for 20-30 min before use.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants- Remove particulates by centrifugation for 10 min at 1500 x g at 4°C and aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

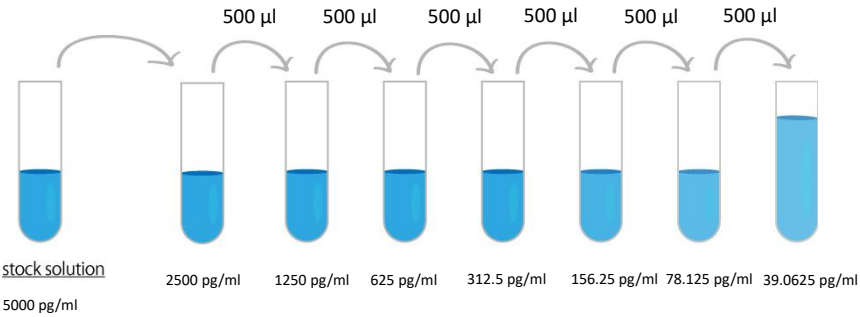
- **1X Wash Buffer:** Dilute **20X** wash buffer with **distilled water** to yield 1X wash buffer, mix thoroughly. (E.g. 30 ml of 20X Wash buffer + 570 ml of distilled water) The dissolved 1X wash buffer is stable for a week at 2°C to 8°C.
- **1X Antibody conjugate**: It is recommended to prepare this reagent immediately prior to use and use it within 1 hours after preparation. Dilute **30X** antibody conjugate concentrate into **Antibody diluent buffer** to yield 1X detection antibody solution, mix thoroughly. (e.g. 30 µl of 30X antibody conjugate concentrate + 870 µl of Antibody diluent buffer)
- **1X HRP-Streptavidin Solution**: It is recommended to prepare this reagent immediately prior to use and use it within 1 hours after preparation. Dilute **30X** HRP-Streptavidin concentrate solution into **HRP-Streptavidin diluent buffer** to yield 1X HRP-Streptavidin Solution buffer, mix thoroughly. (e.g. 30 µl of 30X HRP-Streptavidin concentrate solution + 870 µl of HRP-Streptavidin diluent buffer)

- **Sample**: If the initial assay found samples contain IgG higher than the highest standard, the samples can be diluted with Standard/Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with the diluents buffer before assay.

  (It is recommended to do pre-test to determine the suitable dilution factor).

- **Standards**: Standard solution should be prepared within 1 hours prior to the experiment. Reconstitute the standard with **1 ml** of Standard/Sample diluent to yield a stock concentration of **5000 pg/ml.** Allow the stock standard to sit for at least 5 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The Standard/ Sample diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Standard/ Sample diluent as according to the suggested concentration below: **2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312.5 pg/ml, 156.25 pg/ml, 78.125 pg/ml, 39.0625 pg/ml**.

  Note: The reconstituted standard solutions are best used within 1 hours. The stock standard solution should be stored at 4°C for up to 12 hours, or aliquot & store at-20°C or-80°C for up to 48 hours. Avoid repeated freeze-thaw cycles.



Dilute **Rabbit IgG** standard as according to the table below:

Standard	Rabbit IgG Conc. (pg/ml)	µl of Standard/Sample diluent	µl of standard
S7	2500 pg/ml	500	500 (5000 pg/ml Stock)
S6	1250 pg/ml	500	500 (S7)
S5	625 pg/ml	500	500 (S6)
S4	312.5 pg/ml	500	500 (S5)
S3	156.25 pg/ml	500	500 (S4)
S2	78.125 pg/ml	500	500 (S3)
S1	39.0625 pg/ml	500	500 (S2)
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) 20 min before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards, samples and zero controls (S0, Standard/Sample diluent) into wells. Cover the plate and incubate for 90 minutes at room temperature or overnight at 4°C with gentle shaking.
3. Aspirate each well and wash, repeating the process four times for a total five washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher, keep the Wash Buffer in the wells for 30 sec before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µl of 1X Antibody conjugate into each well, gently tap the plate to mix well. Cover wells and incubate for 60 minutes at 37°C.
5. Aspirate each well and wash as step 3.
6. Add 100 µl of 1X HRP-Streptavidin solution to each well, gently tap the plate to mix well. Cover wells and incubate for 30 minutes at 37°C.
7. Aspirate each well and wash as step 3.
8. Add 100 µl of TMB substrate to each well. Incubate for 15 minutes at 37°C in dark.
9. Add 100 µl of Stop Solution to each well.
10. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

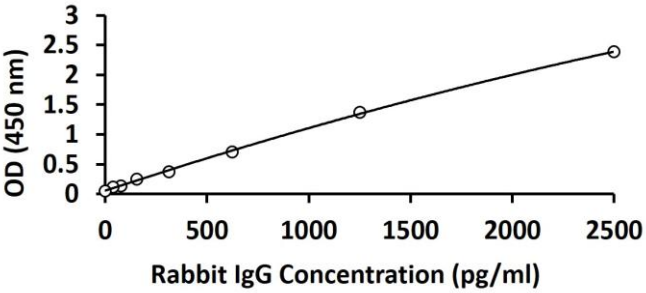
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Rabbit IgG ranged from 39.1- 2500 pg/ml. The mean MDD was 20 pg/ml.

Intra-assay and Inter-assay precision

The CV values of intra-assay was < 10% and inter-assay was < 10%.