User Guide

Human IgG1 ELISA Kit

For Serum, Plasma

RAB0242

Introduction

The human immune system has two main components: the innate system (physical, biochemical, and cellular barriers) and the adaptive immune system (including lymphocytes and immunoglobulins). Immunoglobulins, crucial for vertebrate's humoral immune response against parasites, are composed of two identical heavy (H) chains and two identical light (L) chains linked by inter-chain disulfide bonds. The variable amino acid-rich amino-terminal portions bind antigens, while the constant C-terminal parts bind complement, facilitate placental passage, and attach to cell membranes. Humans have nine immunoglobulin heavy chain isotypes, including IgA (with subclasses IgA1 and IgA2), IgD, IgE, IgM, and IgG (with subclasses IgG1, IgG2, IgG3, and IgG4). IgG predominates in serum, making up 75% of healthy individuals' total serum antibodies, with a molecular weight of around 150 kDa. Four distinct human IgG subgroups (IgG1, IgG2, IgG3, and IgG4) were first identified in the 1960s using polyclonal antisera from animals immunized with human myeloma proteins.

The Human IgG1 ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human IgG1 in serum and plasma. This assay employs an antibody specific for human IgG1 coated on a 96-well plate. Standards and samples are pipetted into the wells and IgG1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human IgG antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IgG1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Storage

The entire kit may be stored at -20°C for up to 1 year from date of shipment. Avoid repeated freeze thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.

Components

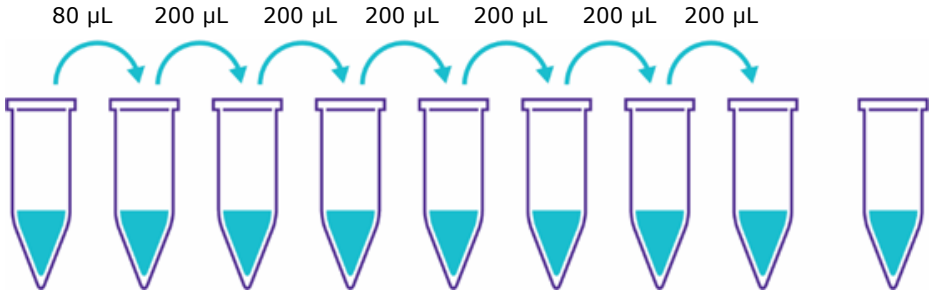
- Human IgG1 Microplate - RAB0242A-EA: 96 wells (12 strips x 8 wells) coated with anti-Human IgG1. Stable in storage 1 month at 4 °C once opened. Return unused wells to the pouch containing desiccant pack, reseal along the edge.
- Human IgG1 Standard Protein - RAB0242C-1VL: 2 vials of Human IgG1. 1 vial is enough to run each standard in duplicate. Stable in storage 1 week at -80 °C once opened.
- Human IgG1 Detection Antibody - RAB0242D-1VL: 2 vials of biotinylated anti-Human IgG1. Each vial is enough to assay half the microplate. Stable in storage 5 days at 4 °C once opened.
- Wash Buffer - RABWASH4: 25 mL of 20X concentrated solution. Stable in storage 1 month at 4 °C once opened.
- HRP-Streptavidin - RABHRP5: 200 µL 2000X concentrated HRP-conjugated streptavidin. Do not store and reuse.
- TMB One-Step Substrate Reagent - RABTMB3: 12 mL of 3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution.
- Stop Solution - RABSTOP3: 8 mL of 0.2 M sulfuric acid.
- 5X Assay Diluent B - RABELADE-15ML (2 bottles): 2 bottles of 15 mL 5X concentrated buffer. Stable in storage 1

Additional Materials Required (But Not Provided)

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µl to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and l liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

Reagent Preparation

1. Bring all reagents and samples to room temperature (18-25 °C) before use.
2. 5X Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.
3. Sample dilution: 1X Assay Diluent B should be used for dilution of serum and plasma samples. The suggested dilution for normal serum/plasma is ~10,000,000-fold. For example, add 1 µL of serum/plasma into a tube with 99 µL 1X Assay Diluent to prepare a 100-fold diluted sample. Mix thoroughly and then pipette 1 µL of prepared 100-fold diluted sample into a tube with 99 µL 1X Assay Diluent to prepare a 10,000-fold diluted sample. Mix thoroughly and then pipette 1 µL of prepared 10,000-fold diluted sample into a tube with 999 µL 1X Assay Diluent to prepare a final 10,000,000-fold diluted sample.
   Note: Levels of IgG1 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.
4. Preparation of standard: Briefly spin a vial of Standard Protein. Add 600 µL 1X Assay Diluent B into the Standard Protein vial to prepare a 300 ng/mL stock standard solution. Dissolve the powder thoroughly by a gentle mix. Add 80 µL IgG1 standard (300 ng/mL) from the vial of Standard Protein, into a tube with 520 µL 1X Assay Diluent B to prepare a 40 ng/mL standard. Pipette 400 µL 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Assay Diluent B serves as the zero standard (0 ng/mL).



		Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	0 Std
Diluent volume	Std + 600 µL	520 µL	400 µL	400 µL	400 µL	400 µL	400 µL	400 µL	400 µL
Concentration	300 ng/mL	40 ng/mL	13.3 ng/mL	4.44 ng/mL	1.48 ng/mL	0.49 ng/mL	0.16 ng/mL	0.05 ng/mL	0 ng/mL

5. If the Wash Buffer (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.
6. Briefly spin the Detection Antibody vial before use. Add 100 µL of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B and used in step 5 of the assay procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 2000-fold with 1X Assay Diluent B.

   For example: Briefly spin the HRP-Streptavidin vial and pipette up and down to mix gently. Add 6 µL of HRP-Streptavidin concentrate into a tube with 12 mL 1X Assay Diluent B to prepare a final 2,000-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Assay Procedure

1. Bring all reagents and samples to room temperature (18-25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µL of each standard (see Reagent Preparation step 4) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.
4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette 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.
5. Add 100 µL of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

Assay Procedure Summary

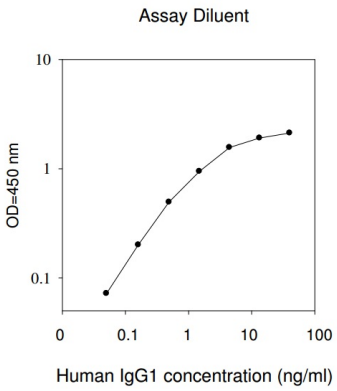
1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µL standard or sample to each well. Incubate 2.5 hours at room temperature.
3. Add 100 µL prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µL prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µL TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µL Stop Solution to each well. Read at 450 nm immediately.

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.



Sensitivity

The minimum detectable dose of Human IgG1 was determined to be 50 pg/mL.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Spiking & Recovery

Recovery was determined by spiking various levels of Human IgG1 into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	79	67-87
Plasma	85	71-97

Linearity

Sample Type		Serum	Plasma
1:2	Average % of Expected	88	82
	Range (%)	77-97	72-94
1:4	Average % of Expected Range	86	81
	Range (%)	75-96	71-92

Reproducibility

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

Specificity

Detect human IgG1. No cross reactivity was found againt IgG2, IgG3, IgG4, IgM, IgA, IgD and IgE

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Briefly centrifuge the standard protein and dissolve the powder thoroughly by gently mixing
Low signal	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
	Too brief incubation times	Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4 °C with gentle shaking. Note: may increase overall signals including background.
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
	Air bubblies in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store your standard at -70 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light.
	Stop Solution	Add stop solution to each well before reading plate

Notice

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RAB0242pis Rev 08/24