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ab100547
Human IgG ELISA Kit

For the quantitative measurement of Human IgG in serum and plasma.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Abcam's IgG Human ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of Human IgG in serum and plasma.

This assay employs an antibody specific for Human IgG coated on a 96-well plate. Standards and samples are pipetted into the wells and IgG 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 IgG bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

2. Protocol Summary

Prepare all reagents, samples and standards as instructed.

Add standard or sample to each well used. Incubate at room temperature

Add prepared biotin antibody to each well. Incubate at room temperature.

Add prepared Streptavidin solution. Incubate at room temperature

Add TMB One-Step Development Solution to each well. Incubate at room temperature.

Add Stop Solution to each well. Read at 450nm immediately.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Amount	Storage Condition (Before Preparation)
IgG Microplate (12 x 8 wells)	96 wells	-20°C
20X Wash Buffer Concentrate	25 mL	-20°C
Recombinant Human IgG Standard	2 vials	-20°C
5X Assay Diluent	2 x 15 mL	-20°C
Biotinylated anti-Human IgG	2 vials	-20°C
4,000X HRP-Streptavidin Concentrate	200 µL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution	8 mL	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 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 1 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.

8. Technical Hints

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- When preparing your standards, it is very critical to briefly spin down the vial first. The powder may drop off from the cap when opening it if you do not spin down. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is a technique we find very effective for thoroughly mixing the standard without too much mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Assay Diluent

5X Assay Diluent should be diluted 5-fold with deionized or distilled water before use.

9.2 1X Wash Solution

If the 20X Wash Concentrate contains visible crystals, equilibrate to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

9.3 1X Biotinylated IgG Detection Antibody

Briefly spin the Biotinylated anti-Human IgG vial before use. Add 100 µL of 1X Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can either be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The detection antibody concentrate must be diluted 80-fold with 1X Assay Diluent prior to use in the Assay Procedure.

9.4 1X HRP-Streptavidin Solution

Briefly spin the 4,000X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. HRP Streptavidin concentrate must be diluted 4,000-fold with 1X Assay Diluent prior to use in the Assay Procedure.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 3 µL of 4,000X HRP-Streptavidin concentrate into a tube with 12 mL 1X Assay Diluent to prepare a final 4,000-fold diluted 1X HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).
- Standard (recombinant protein) should be stored at -20°C or 80°C (recommended at -80°C) after reconstitution.

10.1 Briefly spin the vial of IgG Standard. Prepare the 50 ng/mL **Stock Standard** by adding 400 µL 1X Assay Diluent into the vial (see table below).

10.2 Ensure the powder is thoroughly dissolved by gentle mixing.

10.3 Label tubes #1-7.

10.4 Prepare **Standard #1** by adding 180 µL of the 50 ng/mL Stock Standard, to 420 µL of 1X Assay Diluent into tube #1. Mix thoroughly and gently.

10.5 Pipette 400 µL of 1X Assay Diluent into remaining tubes.

10.6 Prepare **Standard #2** by adding 200 µL Standard #1 to tube #2 and mix thoroughly.

10.7 Prepare **Standard #3** by adding 200 µL Standard #2 to tube #3 and mix thoroughly.

10.8 Using the table below as a guide, prepare further serial dilutions.

10.9 1X Assay Diluent serves as the zero standard (0 ng/mL).

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	180	420	600	50	15
2	200	400	600	15	5
3	200	400	600	5	1.67
4	200	400	600	1.67	0.56
5	200	400	600	0.56	0.19
6	200	400	600	0.19	0.062
7	200	400	600	0.062	0.021
8	0	400	400	0	0

11. Sample Preparation

General Sample Information:

- 1X Assay Diluent should be used for dilution of serum/plasma samples.
- Suggested dilution for normal serum/plasma: 10,000,000-fold.
- For example, add 1 µL of serum/plasma into a tube with 99 µL 1X Assay Diluent B to prepare a 100-fold diluted sample. Mix through 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 through 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.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater *(for reference only; please follow the insert for specific dilution suggested)*	
100x	**10000x**
4 µl sample + 396 µl buffer (100X) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl*
1000x	**100000x**
A) 4 µl sample + 396 µl buffer (100X) B) 24 µl of A + 216 µl buffer (10X) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*	A) 4 µl sample + 396 µl buffer (100X) B) 4 µl of A + 396 µl buffer (100X) C) 24 µl of A + 216 µl buffer (10X) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl*

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Add 100 µL of each standard (see Standard Preparations, section 10) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 4°C with gentle shaking.

13.2 Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with 1X Wash Solution (300 µL) using a multi-channel Pipette or auto washer. 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.

13.3 Add 100 µL of 1X Biotinylated IgG Detection Antibody (Reagent Preparation, section 9.3) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4 Discard the solution. Repeat the wash as in step 13.2.

13.5 Add 100 µL of 1X HRP-Streptavidin solution (see Reagent Preparation section 9.4) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

13.6 Discard the solution. Repeat the wash as in step 13.2.

13.7 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.

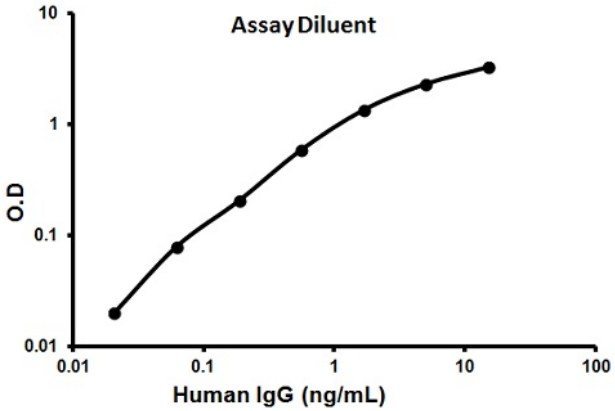
13.8 Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

14. Calculations

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, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



Conc. (ng/mL)	O.D. Assay Diluent
0.021	0.02
0.062	0.079
0.185	0.204
0.556	0.585
1.667	1.338
5	2.30
15	3.257

Figure 1. Example of human IgG standard curve.

16. Typical Sample Values

SENSITIVITY –

The minimum detectable dose of IgG is typically less than 20 pg/mL.

RECOVERY –

Recovery was determined by spiking various levels of Human IgG into Human serum, or plasma. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	116.1	105-123
Plasma	118.1	99-126

Linearity of Dilution

Serum Dilution	Average % Expected Value	Range (%)
1:2	111.3	101-122
1:4	113.2	103-122

Plasma Dilution	Average % Expected Value	Range (%)
1:2	112.5	104-121
1:4	114.4	105-120

PRECISION –

	Intra-Assay	Inter-Assay
CV (%)	<10%	<12%

Please contact our Technical Support team for more information.

17. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

18. Notes

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