Human IgG ELISA Kit

User Manual

For the quantitative measurement of Human IgG

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

CYTO DIAGNOSTICS CYTO GROUP

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Overview

The Human IgG ELISA Kit (EL-003) is a 2-step sandwich ELISA designed for the quantitative measurement of human IgG antibodies in serum, plasma, and cell culture supernatant. The microplate wells supplied with the kit are pre-coated with a target-specific antibody. Upon adding the samples, standards, or controls, the target is captured by the immobilized antibody and is subsequently bound by the enzyme conjugated detector antibody. In the presence of the substrate solution, the enzyme-antibody-target complex develops a measurable, blue-colored end-product which correlates to the concentration of target analyte present in the original sample. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Storage and Stability

Store the kit at 4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, provided the components have not been reconstituted. Observe the storage conditions for individual reconstituted materials in the Reagent Preparation section.

Materials Supplied

Item	Quantity	Storage Condition
Pre-coated Microplate (12 x 8 wells)	96 wells	4°C
10X Wash Buffer 1	50 mL	4°C
1X Sample Diluent	50 mL	4°C
1X Assay Diluent	50 mL	4°C
Human IgG Purified Protein, Lyophilized	1 vial	4°C
200X HRP-Detection Antibody conjugate	150 µL	4°C
1X One-step TMB Substrate	12 mL	4°C
1X Stop Solution	8 mL	4°C
Adhesive Plate Covers	1 count	N/A

Note: For long-term storage, it is recommended the Standard Human IgG is stored at -20°C if the kit is not used immediately or intended to be used at a later date.

Required Materials (not supplied)

The following materials are not included in the kit, but will be required to perform the assay:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes and pipette tips to deliver 2 µL to 1 ml volumes
- Adjustable 1-25 mL pipettes and pump
- 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

Precautions

- Please read the instructions carefully before beginning the assay
- All kit components have been formulated and quality control tested to function successfully as a kit.
- It is not recommended to modify the kit assay protocols. If any modification becomes necessary to meet unique experimental circumstances, it needs to be done by the user's own discretion, conceding the performance of the product outside the kit specified conditions cannot be guaranteed
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- 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.
- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not alter 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.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

Technical Hints

- A 'test' 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.
- Selected kit components are provided in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Ensure plates are properly sealed or covered during incubation steps.
- When preparing your standards, it is critical to briefly centrifuge the vial first. The powder may adhere to the cap and not be included in the standard solution resulting in an incorrect concentration. Be sure to dissolve the powder thoroughly when reconstituting. After adding Sample Diluent to the vial, we recommend waiting for 5 minutes, inverting the tube a few times, then flicking the tube a few times, and centrifuging briefly.
- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be performed at room temperature.
- Samples generating absorbance values higher than the highest standard should be further diluted in the Assay Diluent.
- Avoid foaming or bubbles when mixing components or adding reagents to the assay well
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Complete removal of all solutions and buffers during wash steps.
- Do not let the wells dry out between washes or reagent addition steps.
- Make sure the microplate reader is switched on before starting the experiment.

Reagent preparation

- Equilibrate all reagents to room temperature prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as needed on the day of the experiment.
- Prepare 1X Wash Buffer by adding distilled or deionized water to the provided 10X Wash Buffer. If the 10X Wash Buffer contains visible crystals, equilibrate to room temperature, and mix gently until dissolved.
- Briefly spin down the 200X HRP-Detection Antibody conjugate vial and pipette up and down to mix gently before use. HRP-Detection Antibody conjugate must be diluted 200-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 5 µL of 200X HRP-Detection Antibody conjugate into a tube with 995 µL of 1X Assay Diluent to prepare a final 200-fold diluted 1X HRP-Detection Antibody conjugate solution (do not store the diluted solution for next day use). Mix well.

Standard Preparation

- Briefly spin down the vial of Human IgG Standard. Reconstitute each vial to prepare 100 ng/mL by adding 300 µL of 1X Sample Diluent
- Ensure the powder is thoroughly dissolved by gentle mixing. Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once the Standard has been reconstituted, it should be used right away or aliquoted to store at minus 20°C for a few days for later use. Avoid repeated freeze thawing.
- Prepare serially diluted standards with 1X Assay Diluent immediately prior to use. Always prepare a fresh set of standards for every use.
- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- It is recommended to prepare a seven-point standard curve with a minimum of two replicates using 2-fold serial dilutions, ranging from 62.5 pg/mL to 4000 pg/mL

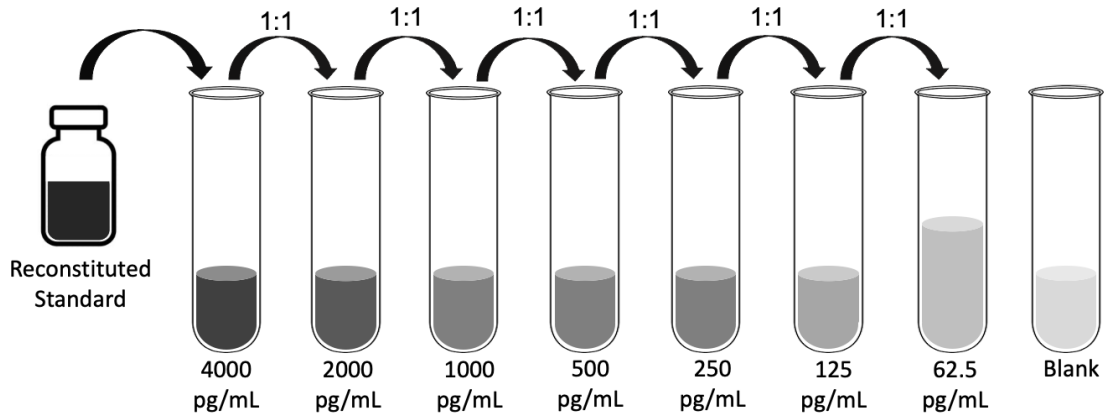

Figure 1: Serial dilution schematic as a guide. One tube will contain only Assay Diluent without Human IgG as the Blank control.

Sample Preparation

- If samples need to be diluted, 1X Assay Diluent should be used for dilution.
- Dilute samples in 1X Assay Diluent to fall within the 62.5 pg/mL - 4000 pg/mL assay range. If concentration of IgG is unknown, try multiple dilutions of the sample in Assay Diluent. The concentration measured from the standard curve needs to be multiplied by the dilution factor.
- Samples generating values higher than the highest standard should be further diluted in the 1X Assay Diluent.
- 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.
- For Cell Culture Supernatant samples, centrifuge collected cell culture media, or supernatant at 2,000 x g for 10 minutes at 4°C to remove debris. Dilute samples in the 1X Assay Diluent provided with the kit. Store undiluted media or supernatant samples at -20°C. Avoid repeated freeze-thaw cycles.

Plate Preparation

- The 96 well plate strips included with the kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be returned to the plate packet and stored at 4°C.
- For statistical significance, it is recommended that each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

Assay Protocol

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate. Avoid foaming or bubbles when mixing or applying samples in the wells.

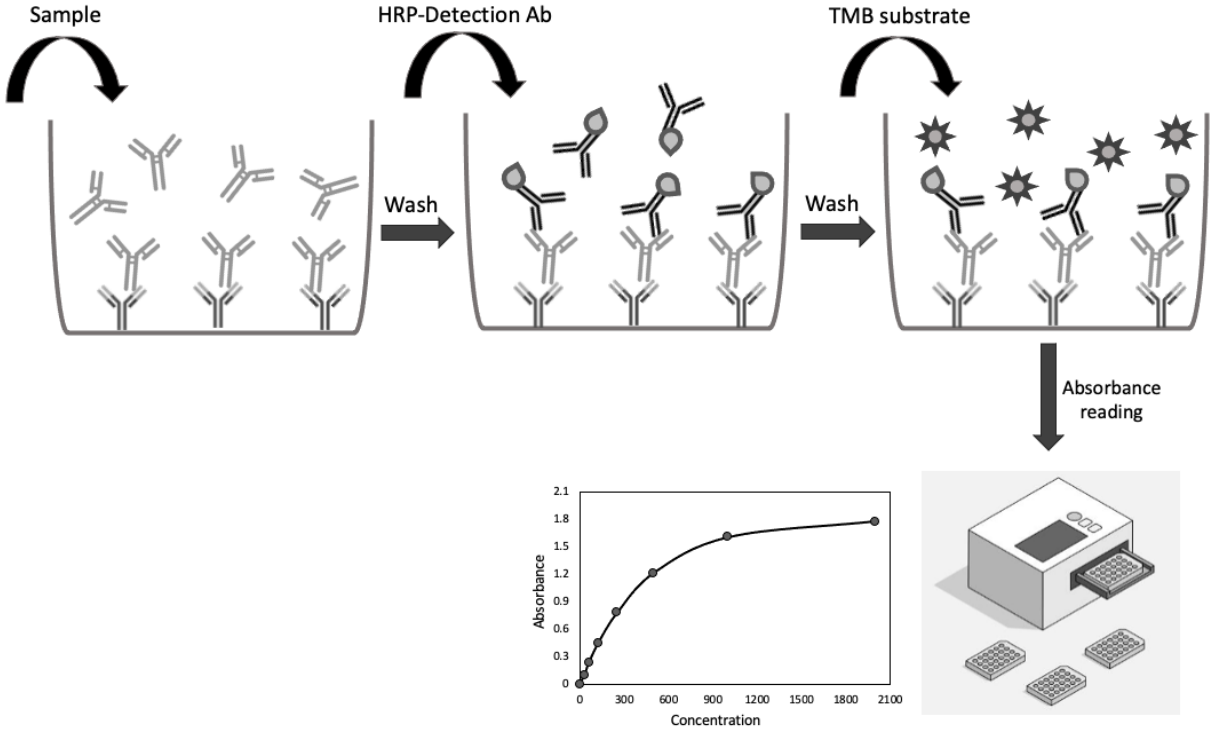

Figure 2: Assay protocol workflow

1. Add 100 µL of each standard (see Standard Preparation section) and sample into appropriate wells. Cover well and incubate for 2 hours at room temperature.
2. Discard the solution and wash 3 times with 1X Wash Buffer 1. Wash by filling each well with 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 1X Wash Buffer 1 by aspirating or decanting. Invert the plate and blot it against clean paper towels.
3. Add 100 µL of 1X HRP-Detection Antibody solution (see Reagent Preparation section) to each well. Cover the plate and incubate for 1 hour at room temperature.
4. Discard the solution. Repeat the wash as in step 2 with one more extra wash step (total 4x).
5. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 20-30 minutes at room temperature in the dark.
   Note: Stop solution may need to be added sooner if blue colour develops rapidly.
6. Add 50 µL of Stop Solution to each well. Read at 450 nm and 540 nm immediately.

Data Analysis

Analyze the data as described below.

- Subtract absorbance readings at 540 nm from the readings at 450 nm.
- Calculate the mean absorbance for each set of duplicate standards, controls, and samples, and subtract the average Blank absorbance value.
- 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 trend line through the standard points.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Typical Data

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

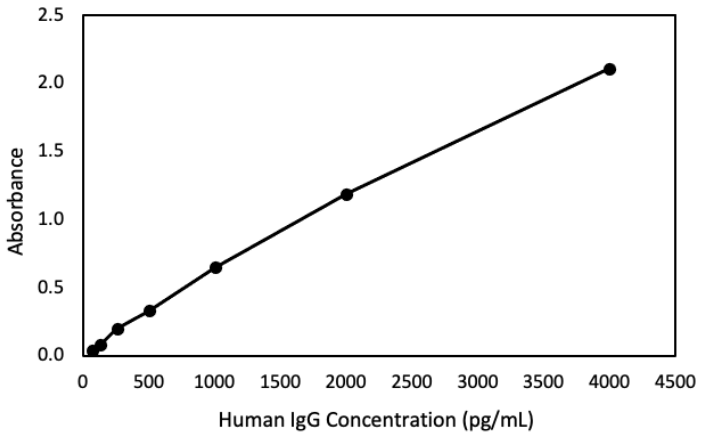

Figure 3. Example of Human IgG standard curve in 1X Assay Diluent. The standard curve was prepared as described in Standard Preparation section.

Typical Sample Values

SENSITIVITY:

The limit of detection (LoD) of Human IgG is typically less than 50 pg/mL.

PRECISION:

	Intra-Assay	Inter-Assay
Coefficient of Variation (%)	<8%	<14%

RECOVERY:

Recovery was determined by spiking various levels of Human IgG into fetal calf serum and hybridoma cell culture media. Mean recovery is as follows:

Sample Type	Average % Recovery	Range (%)
Serum	98.7	96.5 – 100.1
Cell culture media	96.2	90.9 – 99.9

LINEARITY OF DILUTION:

Linearity of dilution was determined by spiking hybridoma cell culture media with high concentration Human IgG and 2-fold dilution with Assay Diluent. Linearity analysis is as follows:

Dilution	Linearity % (cell culture media)
1:2	102.2
1:4	108.8

SPECIFICITY:

All the different IgG tested from other species displayed less than 1% cross-reactivity.

		% Human IgG Signal at A450			
		Human	Rabbit	Mouse	Goat
IgG	8 ng/mL	100	0.65	0.82	0.65
	16 ng/mL	100	0.41	0.48	0.51

Troubleshooting

Problem	Reason	Solution
Poor standard curve	– Inaccurate pipetting – Improper standards dilution	– Check pipettes – Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	– Incubation times too brief – Inadequate reagent volumes or improper dilution	– Ensure sufficient incubation times; change to overnight standard/sample incubation – Check pipettes and ensure correct preparation
Large CV%	– Inaccurate pipetting	– Check pipettes
High background	– Plate is insufficiently washed – Contaminated wash buffer	– Review manual for proper washing technique. If using a plate washer, check all ports for obstructions – Prepare fresh wash buffer
Low sensitivity	– Improper storage of the ELISA kit	– Store the reconstituted protein at -20°C, and all other assay components at 4°C. Keep substrate solution protected from light.

Please contact our Technical Support team for more information.