

Human IgG Easy Quantification Kit

Catalog No: IS002A
IS002B

Size: 1 Plates (1 x 96 tests)
2 Plates (2 x 96 tests)

Application:

The Human IgG Easy Quantification Kit provides a rapid and easy method (one antibody step ELISA) for the quantitative determination of human IgG in cell culture supernatant and serum. The kit includes ready-to-use reagents necessary to analyze up to **89 samples in 30 minutes**. Buffer solutions are **color coded** in order to simplify pipetting steps.

Principle of the Assay:

The method employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific to the gamma heavy chain of human IgG is pre-coated onto the microwells. Samples and standards are pipetted into microwells, and human IgG present in the sample is bound by the capture antibody. Next, a HRP (horseradish peroxidase) conjugated anti-human IgG (H+L) antibody is pipetted and incubated simultaneously with samples. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells. Color develops proportionally to the amount of human IgG in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Specificity:

The method enables the detection of natural human IgG as well as recombinant humanized or fully human monoclonal antibodies. Chimeric (human-mouse) recombinant antibodies are detected with the method, but need a specific standard curve. Cross reactions (determined by ELISA) are < 1% for the following IgG: Mouse, Cow, Horse, Sheep and Swine and is < 2 % for Rabbit IgG. No cross reaction was observed with pure mouse or rat serum, indicating the ability of the method to detect human IgG in *in vivo* studies.

Matrix effect: Culture medium generally does not interfere in the assay, however; it is recommended to evaluate the matrix effect in case of the use of "homemade" culture medium.

Sensitivity:

The detection range is **16 ng/mL to 1000 ng/mL**.

The detection threshold is **4 ng/mL**.

Kit Contents (for 10 x 96 tests):

Item	Description	Quantity
IS002A-P	Pre-coated microplate: 96 microwells coated with anti-human IgG (Gamma) polyclonal antibodies	12 strips of 8 wells
IS002A-A	Human IgG standards Concentrations: 0 – 16 – 62 – 125 – 250 – 500 – 1000 ng/mL (Blue solution)	7 x 0.3 ml
IS002A-B	Sample Diluent (PBS pH 7.4, 1% BSA, 0.1% Tween 20) (Blue solution)	30 mL
IS002A-C	Detection antibody: Peroxidase conjugated anti-human IgG (H+L) polyclonal antibody (Red solution)	12 mL
IS002A-D	Substrate solution (TMB)	12 mL
IS002A-E	Stop solution (2M HCl)	12 mL



Storage and Stability:

- Kit components should be stored at 2-8°C upon arrival. Do Not Freeze!
- Store unopened plate and any unused microtiter strips in the pouch with desiccant.
- After opening, reagents must be handled with care to avoid contamination and should be used within 2 months.

Other Reagents and Supplies Required:

- Pipettes and tips (20-200 µL)
- ELISA plate washer (recommended)
- Microplate reader for absorbance measurements at 450 nm and 620 nm
- Wash solution H₂O, 0.05% Tween 20

NOTE: Other wash solutions may be used, but they have to be tested with the method.

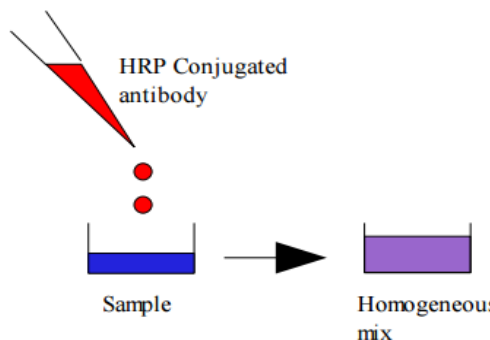
Sample Preparation: Dilute the samples in the sample diluent (Blue).

Recommended dilution factor are indicated in the following table:

Samples	Recommended Dilutions
Cell culture supernatant	1/40
Miniperm, CELLline supernatant	1/1000
Human serum	1/20,000

Assay procedure:

All steps must be performed at room temperature (RT). Bring all the reagents to room temperature 30 minutes before use.

Step 1	Perform the dilution of each sample in the sample diluent buffer (IS002A-A). Serial dilutions may be necessary as recommended previously.
Step 2	Add 20 µL of samples or standards per microwell.
Step 3	<p>Immediately pipette in the same order 100 µL of peroxidase conjugated anti-human IgG (Red solution – IS002A-C). Mix gently until obtaining a homogeneous purple color. Incubate the microwell for 15 minutes at RT.</p>  <p>The diagram shows a pipette tip adding a red liquid labeled 'HRP Conjugated antibody' to a well containing a blue liquid labeled 'Sample'. An arrow points to the resulting 'Homogeneous mix' which is a purple color.</p>
Step 4	After incubation, remove the solution and wash the microwells three times each with 300 µL of the wash solution. An automatic plate washer is recommended.
Step 5	Pipette 100 µL of TMB substrate (IS002A-D) in each well. Incubate for 10 minutes at room temperature.
Step 6	Stop the reaction by pipetting 100 µL of STOP solution (IS002A-E) in the same order as for the TMB distribution.
Step 7	Read the absorbance at 450 nm and 620 nm with a microplate reader.



Calculation of Results:

Validation of the assay: The mean absorbance of the 0 ng/mL standard should be below 0.1 AU (absorbance unit). Maximal absorbance (1000 ng/mL standard) should be around 1.6-2.2 AU, depending of the operating temperature.

Standard curve: Plot the average value (absorbance 450-620 nm) of each standard on the Y axis against their corresponding concentration on the X axis. Software able to generate a cubic spline curve-fit is recommended. The human IgG concentration in the sample can be calculated by interpolation between standard points on the curve.

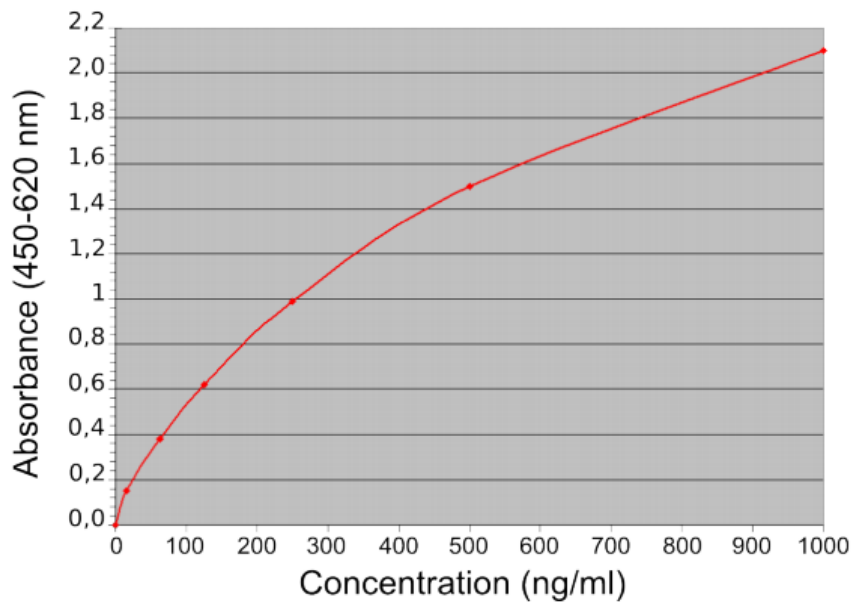
NOTE: It is recommended to repeat the assay at a different dilution factor in the following cases:

- The sample absorbance value is below the first standard.
- The absorbance value is equivalent or higher than the 1000 ng/mL standard.

Hook effect: A hook effect may be observed at IgG concentrations above 5000 ng/mL. In this case, serial dilution of the sample is recommended.

Typical Data:

This standard curve is shown as an example only. A new standard curve should be performed for each series of samples to be tested.



Performance Characteristics:

Intra-assay precision:

Sample	Dilution	Mean concentration (µg/mL)	SD (%)	Number of measures
Supernatant A	1/100	6.8	10.12	10
Supernatant B	1/100	13.4	6.13	10
Supernatant C	1/100	13.9	5.71	10
Supernatant D	1/100	18.8	5.19	10
Supernatant E	1/100	23.5	6.50	10
Supernatant F	1/100	26.9	5.42	10
Supernatant G	1/100	32.7	8.84	10

Inter-assay precision:

Sample	Dilution	SD (%)	Number of measures
Supernatant H	1/250	7.0	30
Supernatant H	1/500	4.6	30
Supernatant H	1/1000	2.4	30

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