HUMAN IMMUNOGLOBULIN QUANTIFICATION KIT

FastELISA®

Catalog Number RDB 3257 (96 tests)

APPLICATION

The human immunoglobulin quantification kit provides a rapid and easy method **(one 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 min. 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 microwells. Samples and standards are pipetted into microwells and human IgG present in the sample are bound by the capture antibody. Then, 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 and 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 are < 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. It is nevertheless recommended to evaluate the matrix effect in case of the use of "home made" culture medium.

SENSITIVITY

The detection range is from **16 ng/ml to 1000 ng/ml**.
The detection threshold is **4 ng/ml**.

STORAGE

All kit components are stable for 12 months when stored at 2-8°C. Do not freeze.
After opening, reagents must be handled with care to avoid contamination and should be used within 2 months.

KIT CONTENTS

Code	Component	Quantity
RDB3257-P	Pre-coated microplates: 96 microwells coated with anti-human IgG (Gamma) polyclonal antibodies	12 strips of 8 wells
RDB3257-Sd	Human IgG standards (Blue solution) Concentrations: 0 – 16 – 63 – 125 – 250 – 500 – 1000 ng/ml	7 x 0.3 ml
RDB3257-D	Sample Diluent (PBS pH7.4, 1% BSA, 0.1% Tween 20) (Blue solution)	30 ml
RDB3257-C	Detection antibody: Peroxidase conjugated anti-human IgG (H+L) polyclonal antibody (Red solution)	12 ml
RDB3257-T	Substrate solution (TMB)	12 ml
RDB3257-St	Stop solution (2M HCl)	12 ml

All the kit components are ready-to-use

ADDITIONAL MATERIAL REQUIRED

- Pipettes and tips (20-200 µl).
- ELISA plate washer (recommended)
- Microplate reader for absorbance measurements at **450 nm** and 620 nm.
- Wash solution: H_2O, 0.05% Tween 20. Other wash solutions may be used but they have to be tested with the method.

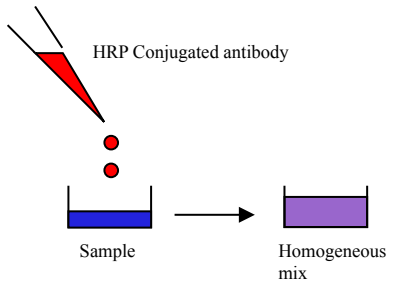
SAMPLE PREPARATION AND STORAGE

If necessary, samples may be stored at -20°C prior to perform the assay.
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, CELLine supernatant	1/1000
Human serum	1/20 000

ASSAY PROCEDURE

All steps must be performed at room temperature (RT). Bring all reagents at RT for 30 min before use.

STEP 1	Perform the dilution of each sample in diluent buffer. Serial dilutions may be necessary as recommended previously.
STEP 2	Add 20 µl of samples or standards per microwell.
STEP 3	 Pipette without delay in the same order 100 µl of peroxidase conjugated anti-human IgG (Red solution). Mix gently until obtaining a homogeneous purple color. Incubate the microwell for **15 min** at RT.
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 into each well. Incubate the plate for **10 min** at RT.
STEP 6	Stop the reaction by pipetting 100 µl of STOP solution in the same order as for 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 to 2.2 AU, depending of the operating temperature.

Standard curve: plot the average value (**absorbance 450-**620) 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:

- If the sample absorbance value is below the first standard.
- If 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. Serial dilution of the sample is then recommended.

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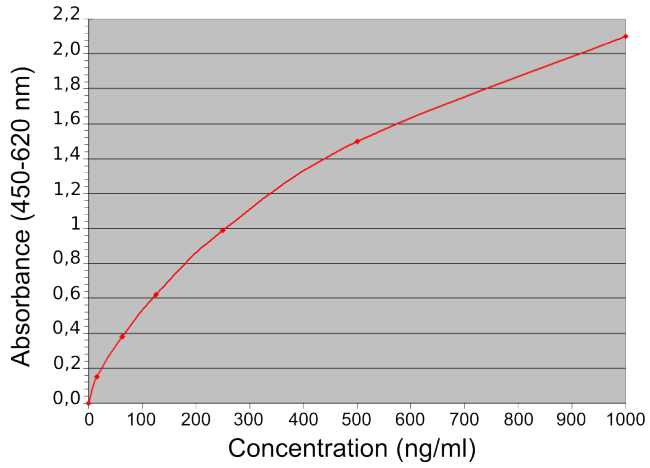
TYPICAL DATA

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



PRECISION

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	6.5	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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