RAT IMMUNOGLOBULIN QUANTIFICATION KIT

RD-Biotech

FastELISA®

Catalog Number RDB 3259-5 (480 tests)

APPLICATION

The rat immunoglobulin quantification kit provides a rapid and easy method **(one step ELISA)** for the quantitative determination of rat IgG in cell culture supernatant and rat ascitic fluid.
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 rat IgG (H+L) is pre-coated onto microwells. Samples and standards are pipetted into microwells and rat IgG present in the sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated anti-rat 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 rat IgG in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

SPECIFICITY

Detection of all rat **subclasses IgG.**

No cross reaction was observed by ELISA with Human and bovine IgG. Cross reactions with mouse IgG are < 8%.

SENSITIVITY

The detection range is **16 ng/ml to 2000 ng/ml**.
The detection threshhold 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
RDB3259(10)-P	Pre-coated microplates: 96 microwells coated with anti-rat IgG (H+L) polyclonal antibodies	120 strips of 8 wells
RDB3259(10)-Sd	Rat IgG standards (Blue solution) Concentrations: 0 – 16 – 125 – 250 – 500 – 1000 – 2000 ng/ml	7 x 1 ml
RDB3259(10)-D	Sample Diluent (PBS pH7.4, 1% BSA, 0.1% Tween 20) (Blue solution)	500 ml
RDB3259(10)-C	Detection antibody: Peroxidase conjugated anti-rat IgG (H+L) polyclonal antibody (Red solution)	120 ml
RDB3259(10)-T	Substrate solution (TMB)	120 ml
RDB3259(10)-St	Stop solution (2M HCl)	120 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: H2O, 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 factors are indicated in the following table:

Samples	Recommended dilutions
Cell culture supernatant	1/100
Miniperm, CELLine supernatant	1/1000
Ascitic fluid	1/10000

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	HRP Conjugated antibody / Sample / Homogeneous mix. Pipette without delay in the same order 100 µl of peroxidase conjugated anti-rat IgG (Red solution). Mix gently until obtaining a homogeneous purple color. Incubate the plate for **15 min** at RT.
STEP 4	After incubation, remove the solution and wash the plate 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 (2000 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 rat 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 2000 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.

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.
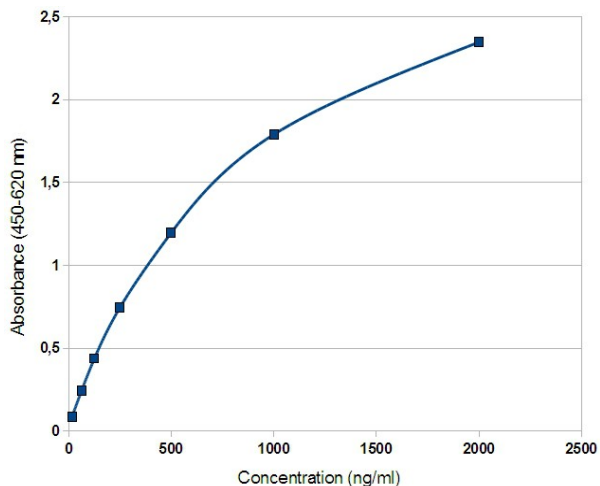
PRECISION

Intra-assay precision

Sample	Dilution	Mean concentration (µg/ml)	SD (%)	Number of measures
Supernatant A	1/10	5.18	2.94	10
Supernatant B	1/10	2.39	3.48	10
Supernatant C	1/10	1.34	4.61	10

Inter-assay precision

Sample	Dilution	SD (%)	Number of measures
Supernatant D	1/100	3.2	30
Supernatant D	1/400	3.59	30
Supernatant D	1/6400	3.13	30