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, 450 or 900 samples** in **30 min** (for RDB-3259-1, RDB-3259-5 or RDB-3259-10 respectively).
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 AND SENSIVITY

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%.

The detection range is **16 ng/ml to 2000 ng/ml**.

The detection threshhold is **5 ng/ml**.

STORAGE

Storage at 2-8°C. After opening, reagents must be handled with care to avoid contamination and should be used within 2 months. Do not freeze.

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RAT IMMUNOGLOBULIN QUANTIFICATION KIT

Catalog number RDB-3259-1 / RDB-3259-5 / RDB-3259-10

Data sheet version : 08/03/2023

FastELISA®

KIT CONTENTS

Code	Component	Quantity 1x96 wells RDB-3259-1	Quantity 5x96 wells RDB-3259-5	Quantity 10x96 wells RDB-3259-10
RDB3259-P	Pre-coated microplates: anti-rat IgG (H+L) polyclonal antibodies	12 strips of 8 wells	60 strips of 8 wells	120 strips of 8 wells
RDB3259-Sd	Rat IgG standards (**Blue solution**) Concentrations (ng/ml): 0 - 16 - 125 - 250 - 500 – 1000 - 2000	7 x 0.3 ml	7 x 0.5 ml	7 x 1 ml
AS31	Sample diluent (**Blue solution**)	30 ml	150 ml	500 ml
RDB3259-C	Detection antibody (**Red solution**)	12 ml	60 ml	120 ml
AS29	TMB	12 ml	60 ml	120 ml
AS40	Stop solution	12 ml	60 ml	120 ml

ADDITIONNEL MATERIEL REQUIRED

Pipettes (20-200 µl).
ELISA plate washer (recommended).
Microplate reader for absorbance measurements at 450 nm and at 620 nm.
Wash solution: H2O + 0.05% Tween 20 or PBS 1X 0.05% Tween 20.

SAMPLE PREPARATION AND STORAGE

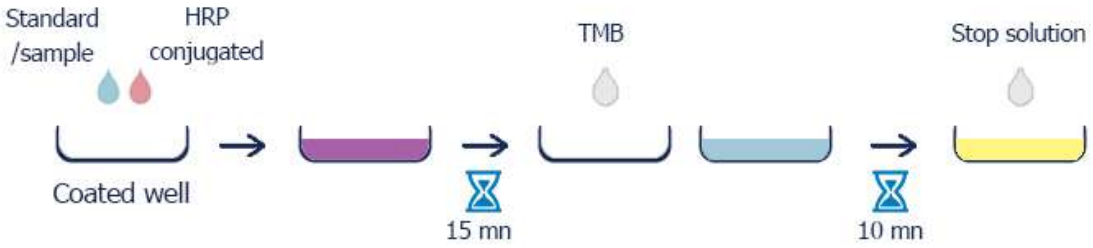
Dilute the samples in the sample diluent to obtain a concentration between 16 ng/ml and 2000 ng/ml.

Samples	Recommended dilutions
Cell culture supernatant	1/100
Miniperm®, CELLine®	1/1000
Ascitic fluid	1/5000

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 (**blue solution**). It is recommended to process the samples in duplicate. Add 20µl of samples or standards per microwell.
STEP 2	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 3	After incubation, remove the solution and wash the plate three times each with **300 µl** of the wash solution.
STEP 4	Pipette 100 µl of TMB substrate into each well. Incubate the plate for **10 min** at RT.
STEP 5	Stop the reaction by pipetting 100 µl of STOP solution in the same order as for TMB distribution.
STEP 6	Read the absorbance at **450 nm** and 620 nm with a microplate reader.



INTERPRETATION AND VALIDATION OF RESULTS

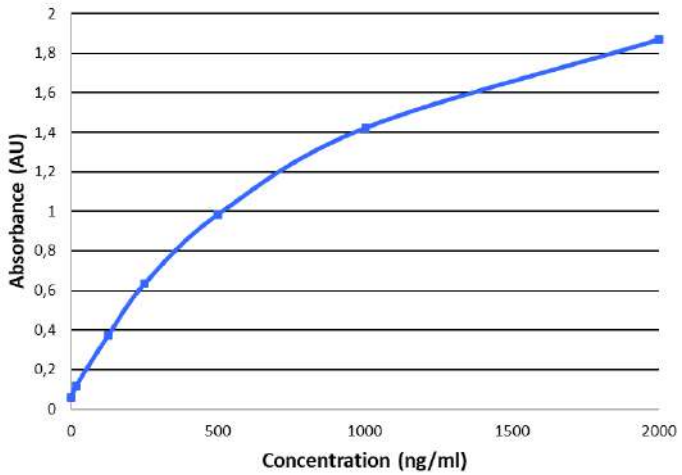
Validation of the assay: The mean absorbance of the 0 ng/ml standard should be below 0.1 AU (absorbance unit). The test does not allow the analysis of undiluted samples with a concentration greater than 2 µg/ml.

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 « Four parameter » 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 or 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 curve:



Concentration (ng/ml)	A450nm-A620nm
0	0.062
16	0.118
125	0.373
250	0.636
500	0.986
1000	1.422
2000	1.869

PRECISION

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

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