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Rat IgG Easy Quantification ELISA Kit

Catalog No: IS004A **Lot No:** TBD **Size:** 1 Plate (1 x 96 tests) **Expiration Date:** TBD
Catalog No: IS004B **Lot No:** TBD **Size:** 10 Plates (10 x 96 tests) **Expiration Date:** TBD

NOTE: this sample protocol is subject to variation by Lot Number. Refer to the protocol inserted in your package for the current lot number specifications and expiration date or contact our technical support at tech@cellsciences.com

Specificity:	Detection of all rat subclasses IgG
Sensitivity:	4 ng/mL
Range:	16 ng/mL to 2000 ng/mL
Sample Type:	Cell culture medium, Miniperm or CELLine supernatant, ascitic fluid
Cross-Reactivity:	No cross reaction was observed by ELISA with Human and bovine IgG. Cross reactions with mouse IgG are < 8%.

Application:
The Rat IgG Easy Quantification ELISA Kit provides a rapid and easy method (one antibody 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 the microwells. Samples and standards are pipetted into microwells, and rat IgG present in the sample is 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 the 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 rat IgG in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Kit Contents (for 1 x 96 tests):

Item	Description	IS004A	IS004B
IS004-P	Pre-coated microplates: 96 microwells coated with anti-rat IgG (H+L) polyclonal antibodies	12 strips of 8 wells	120 strips of 8 wells
IS004-A	Rat IgG standards (Blue solution) Concentrations: 0 – 16 – 125 – 250 – 500 – 1000 – 2000 ng/mL	7 x 300 µL	7 x 1 mL
IS004-B	Sample Diluent (PBS pH 7.4, 1% BSA, 0.1% Tween 20) (Blue solution)	30 mL	500 mL
IS004-C	Detection antibody: Peroxidase conjugated anti-rat IgG (H+L) polyclonal antibody (Red solution)	12 mL	120 mL
IS004-D	Substrate solution (TMB)	12 mL	120 mL
IS004-E	Stop solution (2M HCl)	12 mL	120 mL

All the kit components are ready-to-use.

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Storage and Stability
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.

Other Reagents and Supplies Required:

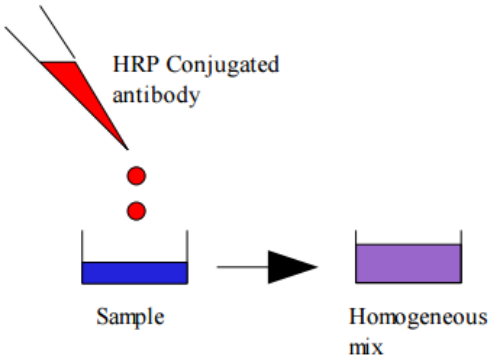
- Pipettes and tips (20-200 µL)
- ELISA plate washer (recommended)
- Microplate reader for absorbance measurements at 450 nm and 620 nm
- Wash solution: H_2O, 0.05% Tween 20
  Note: Other wash solutions may be used, but they have to be tested with the method.

Sample Preparation and Storage:
Samples may be stored at -20 °C prior to performance of 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	1/10,000

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

Step 1	Perform 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	Immediately pipette in the same order 100 µl of peroxidase conjugated anti-rat IgG IS004-C (Red solution). Mix gently until obtaining a homogeneous purple color. Incubate the microwell for 15 minutes 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 IS004-D in each well. Incubate for 10 minutes at room temperature.
Step 6	Stop the reaction by pipetting 100 µL of STOP solution IS004-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.

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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.

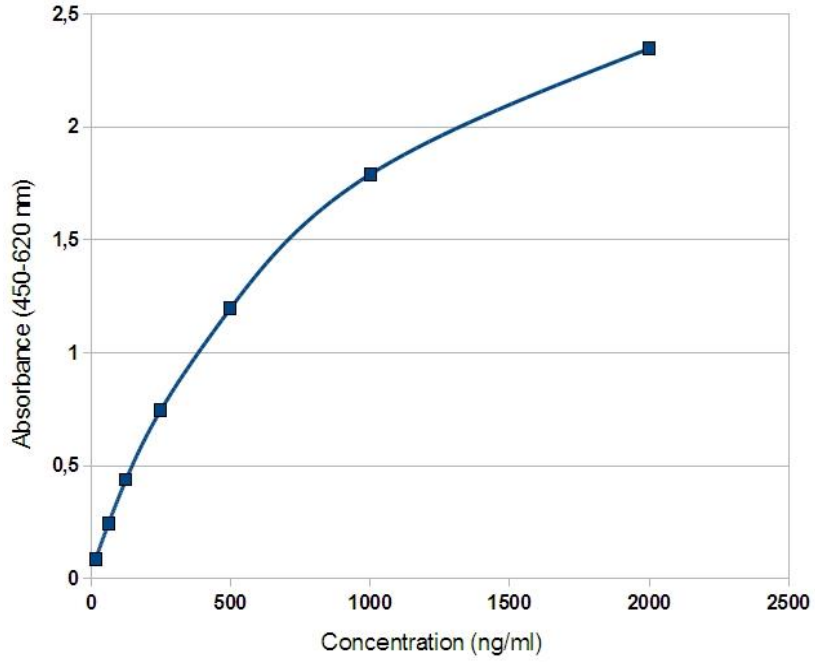
Note: It is recommended to repeat the assay at a different dilution factor in the following cases:

- The sample absorbance value is below the first standard.
- 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. 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.



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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

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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