BOVINE IMMUNOGLOBULIN G QUANTIFICATION KIT

FastELISA®

Catalog Number RDB 3258 (96 tests)

APPLICATION

The bovine immunoglobulin quantification kit provides a rapid and easy method **(one step ELISA)** for the quantitative determination of bovine IgGs in cell culture supernatants and serums (CS, FCS, NBCS...) and contaminating bovine IgGs in batches of purified antibodies produced in vitro.
The kit includes ready-to-use reagents necessary to analyze up to **89 samples** in **45 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 bovine IgG is pre-coated onto microwells. Samples and standards are pipetted into microwells and bovine IgG present in the sample are bound by the capture antibody. Then, an HRP (horseradish peroxidase) conjugated anti-bovine 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 bovine 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 bovine IgG.
No cross reaction was observed with the following species: Mouse and Human.

SENSITIVITY

The detection range is **10 ng/ml to 2000 ng/ml**.
For antibodies with a concentration of 1 mg/ml, the kit allows the detection of bovine IgG contaminations of about 20 ppm.

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
RDB3258-P	Pre-coated microplates: 96 microwells coated with anti-bovine IgG polyclonal antibodies	6 strips of 16 wells
RDB325-Sd	Bovine IgG standards (Blue solution) Concentrations: 0 – 31 – 125 – 250 – 500 – 1000 – 2000 ng/ml	7 x 0.3 ml
RDB3258-D	Sample Diluent (PBS pH7.4, 1% BSA, 0.1% Tween 20) (Blue solution)	30 ml
RDB3258-C	Detection antibody: Peroxidase conjugated anti-bovine IgG (H+L) polyclonal antibody (Red solution)	12 ml
RDB3258-T	Substrate solution (TMB)	12 ml
RDB3258-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: PBS, 0.05% Tween 20.

SAMPLE PREPARATION

Dilute the samples in the sample diluent (Blue).

To quantify bovine IgG in a **fetal calf serum**:
- depleted serum: dilute the sample to 1/4
- non depleted serum: dilute the sample to 1/200

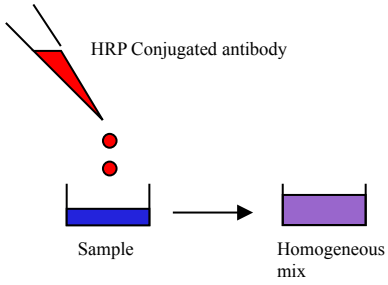
To quantify bovine IgG **in a calf serum**:
- dilute the sample to 1/20000

To quantify bovine IgG in a **batch of antibodies produced in vitro**:
- with depleted serum: dilute the sample to get the purified antibodies at the concentration of 1mg/ml
- with non depleted serum: dilute the sample to get the purified antibodies at the concentration of 50 µg/ml

NB: The dilutions are recommended dilutions, if the absorbance values which are obtained are not in the range of the standard curve, repeat the assay by modifying the dilution of the sample to be analyzed.

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-bovine IgG (Red solution). Mix gently until obtaining a homogeneous purple color. Incubate the plate for **30 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 or a 4 parameters curve is recommended.
The bovine 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.

Example: One antibody at a concentration of 1mg/ml is tested at 16µg/ml of bovine IgG, the antibody is thus contaminated by 1,6% of bovine IgG.
In certain cases it can be necessary to re-calibrate the range of standards with regards to the standards of the laboratory usually used.

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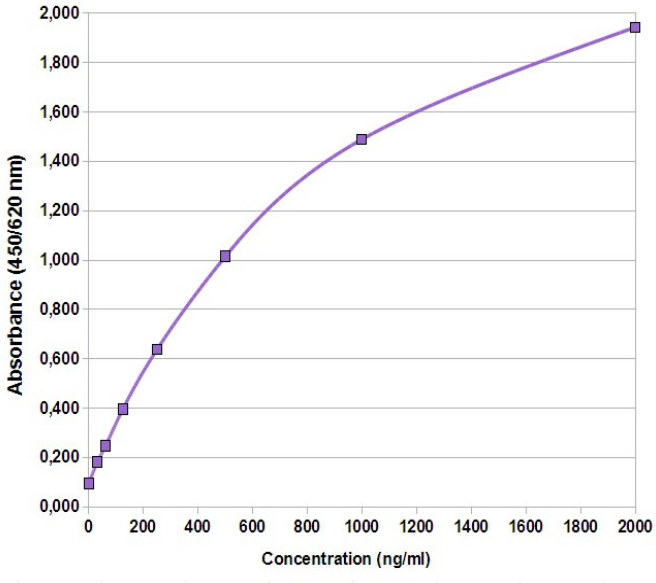
TYPICAL DATA

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
Fetal Bovine Serum	1/100	38.00	6.5	32
Fetal Bovine Serum	1/200	44.17	7.6	32
Fetal Bovine Serum	1/400	41.55	10.8	32

Inter-assay precision

Sample	Dilution	Mean concentration (µg/ml)	SD (%)	Number of measures
Purified antibody A	1/16	7.3	5.87	14
Purified antibody B	1/128	7.7	5.89	14
Bovine Antibody	1/100	1.2	4.3	16

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