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, 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, 450 or 900 samples** (for RDB-3258-1, RDB-3258-5 or RDB-3258-10 respectively) 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 AND SENSIVITY

The method enables the detection of bovine IgG.

No cross reaction was observed with the following species: Mouse and Human.

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

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.

KIT CONTENTS

Code	Component	Quantity
RDB3258-P	Pre-coated microplates: anti-bovine IgG polyclonal antibodies	12 strips of 8 wells
RDB3258-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 (**Blue solution**)	30 ml
RDB3258-C	Detection antibody (**Red solution**)	12 ml
AS29	Substrat solution (TMB)	12 ml
AS40	STOP solution	12 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

Dilute the samples in the sample diluent.

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

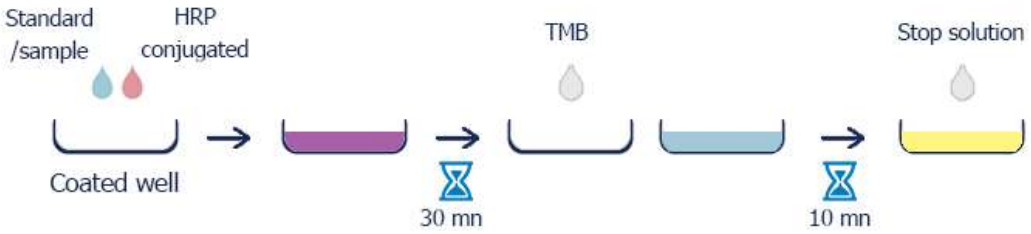
To quantify bovine IgG in a batch of antibodies produced in vitro:
- with depleted serum: dilute the sample to get the purified antibodies at the concentration of 1mg/ml

- with non depleted serum: dilute the sample to get the purified antibodies at the concentration of 50 µg/ml

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-bovine IgG (**Red solution**). Mix gently until obtaining a homogeneous **purple color**. Incubate the plate for **30 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 0.002 ng/ml.

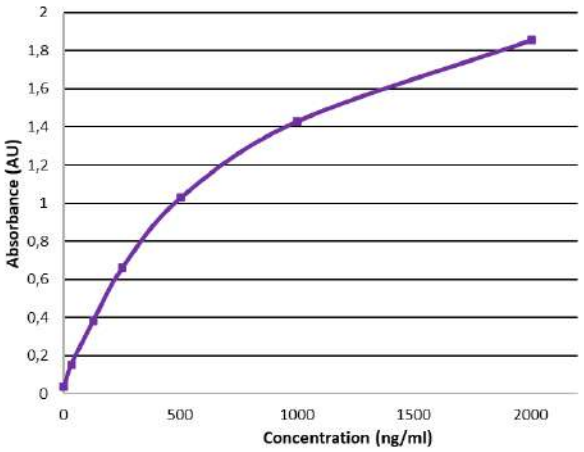
Standard curve: plot the average value (absorbance 450-620 nm) of each standard on the Y axis against their corresponding concentration on the X axis. Software able to generate a « Four parameter » curve-fit is recommended.
The bovine IgG concentration in the sample can be calculated by interpolation 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 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.

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.

Typical curve:



Concentration (ng/ml)	A450nm-A620nm
0	0.041
31	0.155
125	0.384
250	0.663
500	1.030
1000	1.431
2000	1.856

PRECISION

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
Bovin antibody	1/100	1.2	4.3	16

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