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Bovine IgG Easy Quantification ELISA Kit

Catalog No: IS005 **Quantity**: 1 x 96 tests

The Bovine IgG Easy Quantification Kit provides a rapid and easy method (one antibody step ELISA) for the quantitative determination of bovine IgG in cell culture supernatant and serum. 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 the microwells. Samples and standards are pipetted into microwells, and bovine IgG present in the sample is bound by the capture antibody. Next, a HRP (horseradish peroxidase) conjugated anti-bovine 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 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 all bovine IgG.
No cross reaction was observed with the following species: Mouse and Human.

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

Kit Contents (for 1 x 96 tests):

Item	Description	Quantity
IS005-P	Pre-coated microplates: 96 microwells coated with anti-bovine IgG polyclonal antibodies	6 strips of 16 wells (2 wells x 8 wells)
IS005-Sd	Bovine IgG standards (Blue solution) Concentrations: 0 – 31 – 125 – 250 – 500 – 1000 – 2000 ng/ml	7 x 0.3 ml
IS005-D	Sample Diluent (PBS pH 7.4, 1% BSA, 0.1% Tween 20) (Blue solution)	30 ml
IS005-C	Detection antibody: Peroxidase conjugated anti-bovine IgG (H+L) polyclonal antibody (Red solution)	12 ml
IS005-T	Substrate solution (TMB)	12 ml
IS005-St	Stop solution (2M HCl)	12 ml

All the kit components are ready-to-use.

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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.

Additional Materials 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
  Note: Other wash solutions may be used, but they have to be tested with the method.

Sample Preparation:
Dilute the samples in the sample diluent (Blue).
To quantify bovine IgG in fetal calf serum:

- Depleted serum: Dilute the sample to 1/4 in the diluent.
- Non depleted serum: Dilute the sample to 1/200.

To quantify bovine IgG in calf serum:

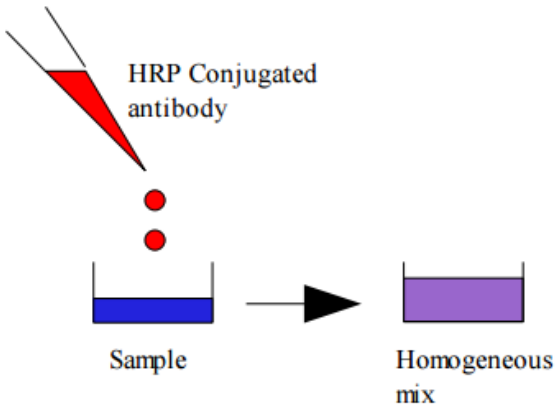
- Dilute the sample to 1/20,000.

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 1 mg/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 that 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 the reagents to room temperature 30 min before use.

Step 1	Perform the dilution of each sample in diluent buffer. Serial dilutions may be necessary as previously recommended.
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-bovine IgG (Red solution). Mix gently until obtaining a homogeneous purple color. Incubate the plate for 30 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.

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Step 5	Pipette 100 µl of TMB substrate in each well. Incubate for 10 minutes at room temperature.
Step 6	Stop the reaction by pipetting 100 µl of STOP solution in the same order as for the 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 absorbance unit. Maximal absorbance (2000 ng/ml standard) should be around 1.6 to 2.2 units, depending of the operating temperature.

Standard curve: Plot the average value (absorbance 450 minus absorbance 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 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:

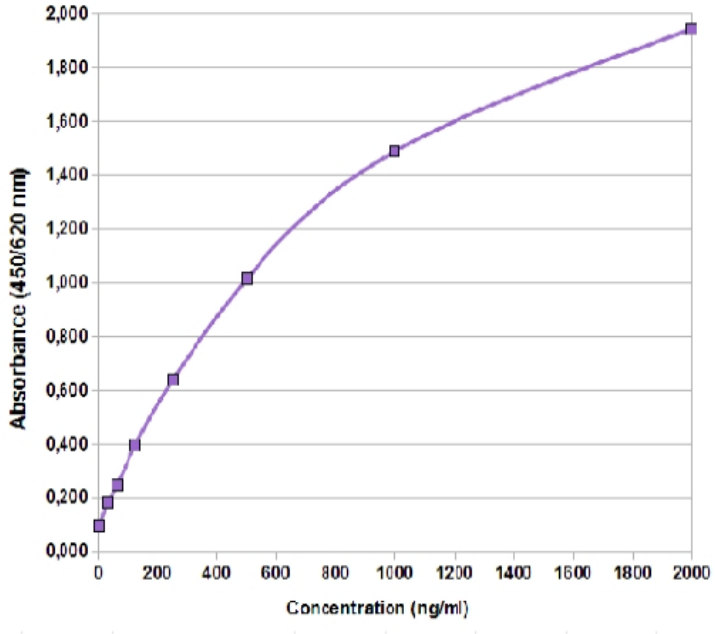
- The sample absorbance value is below the first standard.
- The absorbance value is equivalent or higher than the 2000 ng/ml standard.

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.

Example: One antibody at a concentration of 1mg/ml is tested at 16 µg/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 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
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

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

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