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Bovine Immunoglobulin Quantification Kit

Catalog No: IS005 **Size:** 1 plate (1 x 96 tests)

Specificity:	Detection of bovine IgG.
Sensitivity:	For antibodies with a concentration of 1 mg/ml, the kit allows the detection of bovine IgG contaminations of about 20 ppm.
Detection Limit:	10 ng/ml
Range:	10 ng/mL to 2000 ng/mL
Sample Type:	Cell culture medium, Miniperm or CELLine supernatant, ascitic fluid
Cross-Reactivity:	No cross reaction was observed with Mouse or Human.

Application:
The Bovine Immunoglobulin Quantification Kit provides a rapid and easy method (one antibody 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 minutes. 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 IgGs 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. 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.

Kit Contents (for 1 x 96 tests):

Item	Description	Quantity
IS005-P	Pre-coated microplate: 96 microwells coated with anti-bovine IgG polyclonal antibodies	1 x 6 strips of 16 wells (2 wells x 8 wells)
IS005-A	Bovine IgG standards Concentrations: 0 – 31 – 125– 250 – 500 – 1000 – 2000 ng/mL (Blue solution)	7 x 300 µL
IS005-B	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-D	Substrate solution (TMB)	12 mL
IS005-E	Stop solution (2M HCl)	12 mL

All the kit components are ready-to-use. Once opened, use components within 2 months.

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Storage and Stability:

- Kit components should be stored at 4°C upon arrival. **Do Not Freeze!**
- Store unopened plate and any unused microtiter strips in the pouch with desiccant.
- After opening, reagents must be handled with care to avoid contamination and used within 2 months.

Other Reagents and Supplies Required:

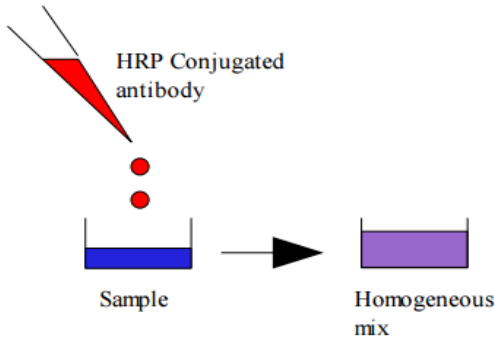
- Pipettes and tips (20-200 µL)
- ELISA plate washer (recommended)
- Microplate reader for absorbance measurements at 450 nm and 620 nm
- Wash solution PBS, 0.05% Tween 20

Sample Preparation: Dilute the samples in the sample diluent (Blue).

Recommended dilution factor are indicated in the following table:

Samples	Recommended Dilutions
Fetal calf serum, depleted	1/4
Fetal calf serum, non-depleted	1/200
Calf serum	1/20,000
Batch of antibodies produced in vitro, with depleted serum	Dilute sample to get the purified antibodies to concentration of 1 mg/mL
Batch of antibodies produced in vitro, with non-depleted serum	Dilute sample to get the purified antibodies to concentration of 50 µg/mL

Assay procedure: Bring all the reagents to room temperature 30 minutes before use.

Step 1	Perform the dilution of each sample in the sample diluent buffer (IS005-B). 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-bovine IgG (Red solution – IS005-C). Mix gently until obtaining a homogeneous purple color. Incubate the microwell for 30 minutes at RT. 
Step 4	After incubation, remove the solution and wash the microwells three times each with 300 µL of the wash solution. An automatic plate washer is recommended.
Step 5	Pipette 100 µL of TMB substrate (IS005-D) in each well. Incubate for 10 minutes at room temperature.
Step 6	Stop the reaction by pipetting 100 µL of STOP solution (IS005-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 1.6-2.2 AU, depending of the operating temperature.

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 cubic spline curve-fit is recommended. The bovine IgG concentration in the sample can be calculated by interpolation between standard points on the curve.

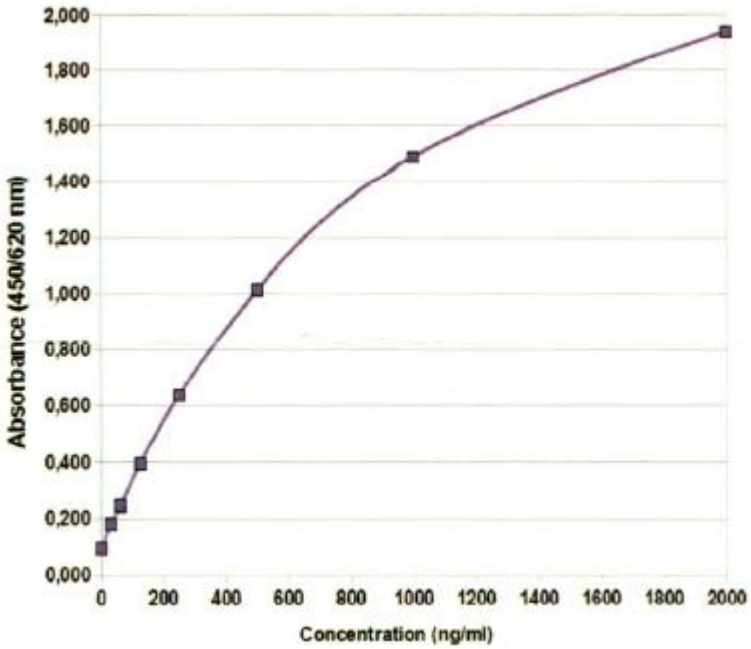
NOTE: It is recommended to repeat the assay at a different dilution factor in the following cases:

- 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.

Typical Data:

This standard curve is shown as an example only. A new stnadard curve should be performed for each series of samples to be tested.



Performance Characteristics:

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	44.7	7.6	32
Fetal Bovine Serum	1/400	41.55	10.8	32

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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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