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ab273152
Bovine IgG ELISA kit - 45 minutes

For the quantitative determination of bovine IgGs in cell culture supernatants and serums, and contaminating bovine IgGs in batches of purified antibodies produced in Vitro.

This product is for research use only and is not intended for diagnostic use.

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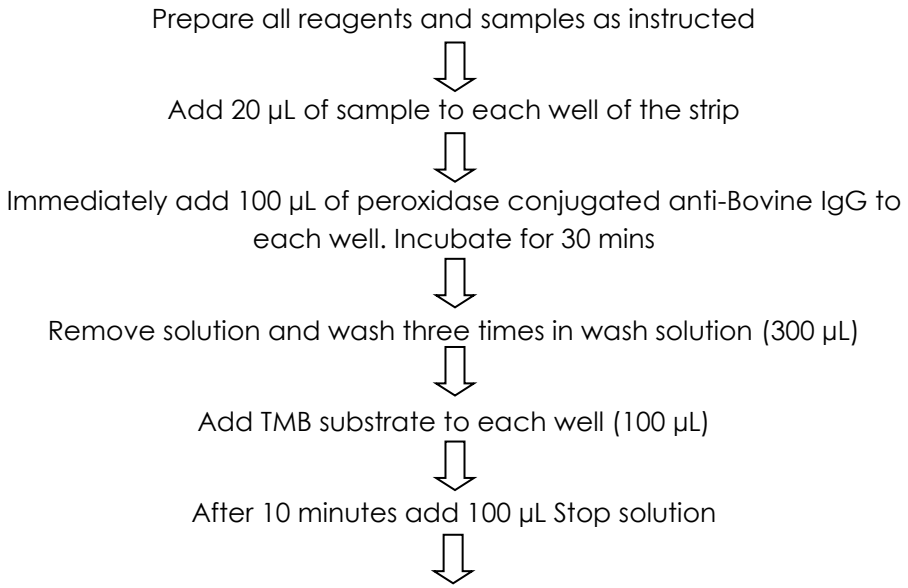
1. Overview

The Bovine Immunoglobulin G ELISA Kit (ab273152) 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 89 samples in less than 45 minutes.

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.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon receipt. Kit has a storage time of 12 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	1 x96 tests	10 X 96 tests	Storage Condition
Pre-coated microwells strips	6 strips of 16 microwells	60 strips of 16 microwells	+4°C
Sample Diluent	30 mL	500 mL	+4°C
Detection antibody	12 mL	120 mL	+4°C
TMB Substrate	12 mL	120mL	+4°C
Stop Solution	12 mL	120 mL	+4°C
Bovine IgG Standards 0 – 31 – 125 – 250 – 500 – 1000 – 2000 ng/ml	7 X 0.3 mL	7 X 1 mL	+4°C

Note: This ELISA kit will soon contain the "Easy View" colored reagents. The Standard diluent buffer will now be red, and the Streptavidin-HRP Diluent will be green. Please note that while stock lasts you may still receive colorless diluents. This change does not impact the results provided by the kit or the assay procedure.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- ELISA plate washer
- Wash solution (H_20, 0.05% Tween 20)
- Standard microplate reader - capable of reading absorbance at 405 nm and 620nm.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample and reagent.
- Pipette samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

– Equilibrate all reagents to room temperature (25°C or 37°C) prior to use. The kit contains enough reagents for 100 assays.

All reagents are supplied ready to use.

10. Sample Preparation

Dilute the samples in dilution buffer. Recommended dilution factor are indicated in the following table:

Samples	Recommended dilutions
In a fetal calf serum	Depleted serum: 1/4 Non depleted serum: 1/200
In a calf serum	1/20000
Produced in vitro	Depleted serum: 1 mg/ml Non-depleted serum: 50 µg/ml

11. Assay Procedure

– Equilibrate all prepared reagents to desired assay temperature prior to use.

11.1 Transfer 20 µL of diluted samples or standard in each well of the strip

11.2 Immediately add 100 µL of Detection antibody to each well. Mix gently until obtaining a homogeneous purple color.

11.3 Incubate at room temperature for 30 minutes.

11.4 After incubation, remove the solution and wash the wells three times with 300 µL of wash solution.

11.5 Add 100 µL of TMB substrate to each well. Tap plate briefly to mix. Incubate for 10 minutes at room temperature.

11.6 After incubation add 100 µL of stop solution to each well.

11.7 Results can be seen directly or read the absorbance with a microplate reader at 450 nm and 620 nm.

12. Calculations

12.1. For each well, subtract the Absorbance readings at 620 nm from the Absorbance readings at 450 nm.

12.2. Calculate the average corrected absorbance values for each standard and sample.

12.3. Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding standard concentration on the horizontal axis.

Note: Use the best curve fit for the generated standard (linear, polynomial, 4PL, 5PL).

12.4. The Bovine IgG concentration in the sample can be calculated by interpolation between standards points on the curve.

Validation of the assay: The mean absorbance of the 0 ng/ml standard should be below 0.1 AU (absorbance unit).

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 0 ng/ml standard.

If the absorbance value is equivalent to or higher than the 2000 ng/ml standard.

Hook effect: A hook effect may be observed at IgG concentrations above 5000 ng/ml. It is advised to prepare a serial dilution of the samples. See section "10. Sample Preparation" for recommendations.

13. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

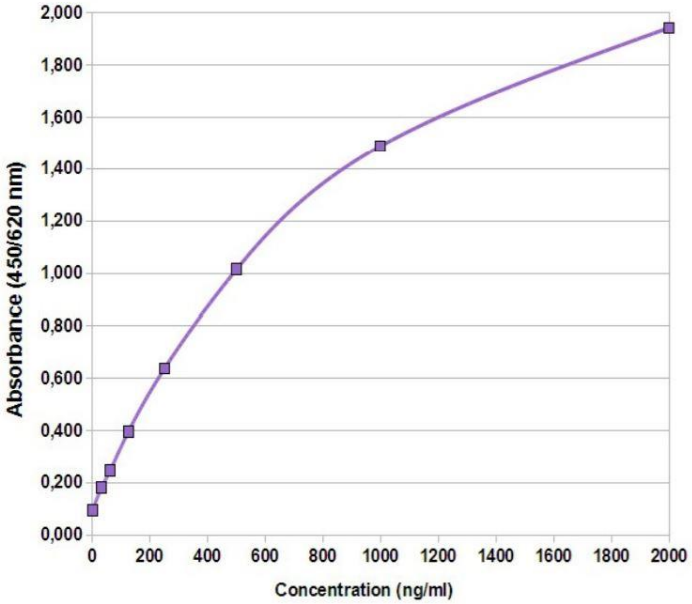


Figure 1. Example of Bovine IgG ELISA kit Standard curve

14. Performance Characteristics

- **Precision**

Intra-Assay				
Sample	Dilution	Mean Concentration (µg/ml)	SD (%)	No of measures
Foetal Bovine Serum	1/100	38.00	6.5	32
Foetal Bovine Serum	1/200	44.17	7.6	32
Foetal Bovine Serum	1/400	41.55	10.8	32

Inter-Assay				
Sample	Dilution	Mean Concentration (µg/ml)	SD (%)	No 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

15. Notes

Technical Support

For all technical or commercial enquiries please go to:

https://www.abcam.com/en-us/contact-us

https://www.abcam.cn/contact-us (China)

https://www.abcam.co.jp/contact-us (Japan)