## Bovine IgG ELISA PROCEDURE SUMMARY

**Total Assay Time - 105 min. (60+30+15)**

| | |
|---|---|
| | Allow **all** reagents to reach room temp.; arrange and label required # of strips. Dilute wash buffer 1:100 and Sample Diluent 1:20 with water. Dilute serum samples (1:60-100k) and HRP conjugate (1:100) with 1x sample diluent. **Do not dilute standards**. |
| **Step 1** | Pipet **20 ul** of pre-diluted **standards** and diluted samples (1:60-100k) into appropriate wells. Add 80ul of 1x Sample diluent. Mix gently, cover the plate and incubate for **60 min** at room temp |
| **Step 2** | Aspirate and wash 3 times with 1x wash solution. Dispense 100 ul of antibody-enzyme conjugate to each well. Mix gently, cover the plate and incubate for 30 min at room temp. |
| **Step 3** | Aspirate and **wash 5 times** with 1x wash solution. Dispense **100 ul of TMB substrate Solution**. Mix gently, cover the plate and incubate for **15 min** at room temp. Blue color develops |
| **Step 4** | Pipette 100 ul stop solution into each well. Blue color turns yellow. Measure Absorbance at 450 nm. |

**CHECK LIST (Check each box after completing each of the above steps)**

| | Step 1 | Step2 | Step3 | Step4 |
|---|---|---|---|---|
| Start time<br>End Time | | | | |

**KIT PROFILE**

**Date received**: **Cat #** 8010 **Lot #** _____ **Exp.** _____

**Date kit opened** _____ **Technician:** _____

**Date used**: **# Strips used** _ **# Remaining** _____

**Date used**: **# Strips used** _ **# Remaining** _____

**Remarks** _____

_____

_____

_____



*Instruction Manual No. M-8010*

# Bovine Serum IgG
# ELISA Kit Cat. # 8010

## For Quantitative Determination of IgG In Bovine Serum

**Bovine IgG ELISA KIT Cat. No. 8010**

| Kit Components, 96 tests | Cat # |
|---|---|
| Anti-Bovine IgG coated strip plate (8 wells x 12 strips) | 8011 |
| Bovine IgG Std. A (0 ng/ml), 0.25 ml | 8012 |
| Bovine IgG Std. B (25 ng/ml), 0.25 ml | 8013 |
| Bovine IgG Std. C (50 ng/ml), 0. 25 ml | 8014 |
| Bovine IgG Std. D (100 ng/ml), 0. 25 ml | 8015 |
| Bovine IgG Std. E (250 ng/ml), 0. 25 ml | 8016 |
| Bovine IgG Std. F (500 ng/ml), 0. 25 ml | 8017 |
| Bovine IgG Std. G (1000 ng/ml), 0. 25 ml | 8018 |
| Sample Diluent (20x), 10ml | SD-20B |
| Wash Buffer (100X), 10 ml | WB-100 |
| Anti-Bovine IgG-HRP Conjugate (100x), 0.12 ml | 8019 |
| TMB Substrate, 12 ml | 80091 |
| Stop solution, 12 ml | 80101 |
| Instruction Manual | M-8010 |

## Introduction

Immunoglobulins belong to a large group of related glyco-proteins that make up approximately 20% of the serum proteins. The serum immunoglobulins react with antigens and confer immunity to individuals. All immunoglobulins share the basic structure of: 2 identical heavy chains joined by disulfide bonds to 2 identical light chains. Both the heavy (H) chains and the light (L) chains are divided into constant and variable regions. The constant regions have similar amino acid composition between all the immunoglobulin classes while the variable regions encompasses about 110 amino acids characterized by a high degree or sequence variability.
Its H-chain type, based on the amino acid sequence, determines the classes of an immunoglobulin. There are 5 types of H-chains that correspond to the following immunoglobulin classes: IgG, IgA, IgM, IgD, and IgE.
IgG is further subdivided into 4 subclasses with ~95% homology. There are 2 subclasses of IgA. IgG and IgA exists in serum as a monomer consisting of a single 4-polypeptide unit. IgM exists in serum as a pen tamer. IgA may also polymerize to form polymers containing 2-5 structural units.
It is important to measure the level of immunoglobulins in serum for Antibody deficiency conditions, such as Primary hypogammagobulinaemia, or other immune deficiency diseases such as AIDS. When evaluating patients with recurrent infections, suspected immunodeficiency, allergic disease and many other conditions, it may be necessary to quantify the levels of immunoglobulins.

ADI's Bovine IgG ELISA kit is a highly sensitive sandwich type assay for the measurement of IgG in serum. The assay can be adapted to measure Bovine IgG in other biological fluids such as plasma, urine, culture medium etc.



## PERFORMANCE CHARACTERISTICS

**1. Detection limit**- Based on 7 replicate determinations of the zero standards, the minimum IgG concentration detectable using this assay is 15 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

**2. Expected Values**: A limited testing of 4 adult Bovine serum samples values of 16.8 - 25.6 mg/ml (average 20.4 mg/ml).

**3. Specificity:** The antibodies used in this kit are specific for IgG and show no significant reactivity with bovine IgA, IgM or other serum proteins.

**4. Species Crossreactivity**
Cross reactivity was tested with the following animal serum at dilutions of 1:10k: Significant crossreactivity was observed with Rabbit (2.2%) serum. Human, Rat, Mouse, Goat, Sheep, PIG, FBS, Guinea Pig, Hamster, Monkey, and Chicken serum had less than 1% crossreactivity.

**Other ELISA kits are available from ADI (**complete list at the web site)

**Human**: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgE, IgG1, IgG4, Insulin, NSE, CA125, CA199, CA242, PAP, Resistin, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, E2, testosterone, progesterone etc).
**Monkey**: IgM, IgG, IgA, CRP
**Rat**: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein
**Mouse**: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF,
**Chicken**: IgG, IgM, IgY, Ovalbumin
**Rabbit**: CRP, IgG
**Bovine**: Albumin, IgG, IgM, Lactoferrin, Transferrin
**Pig**: Albumin, IgG, IgM,
**Dog:** CRP, IgG, IgM
**Cat**: IgG, IgM
**Goat:** IgG
**Sheep:** IgG
**Turkey**: IgG

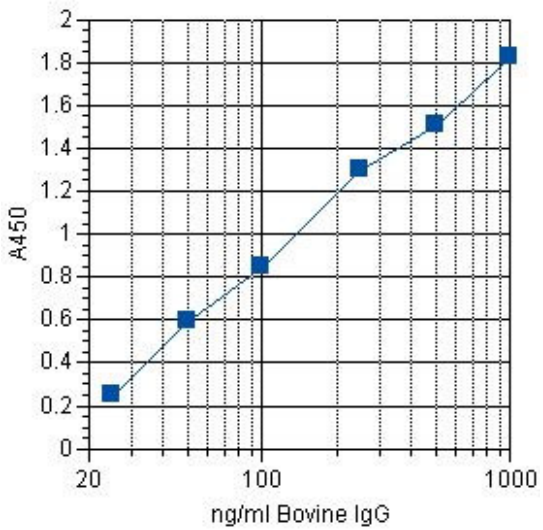
For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

## WORKSHEET OF TYPICAL ASSAY

| Wells | Stds/samples | *Mean $A_{450\ nm}$ | Calculated Concn |
|---|---|---|---|
| A1, A2 | **Std. A** (0 ng/ml) | 0.025 | |
| B1, B2 | **Std. B** (25 ng/ml) | 0.25 | |
| C1, C2 | **Std. C** (50 ng/ml) | 0.59 | |
| D1, D2 | **Std. D** (100 ng/ml | 0.77 | |
| E1, E2 | **Std. E** (250 ng/ml) | 1.3 | |
| F1, F2 | **Std. F** (500 ng/ml) | 1.51 | |
| G1, G2 | **Std. G** (1000 ng/ml) | 1.82 | |
| H1, H2 | **Sample 1 (1:80K)** | 1.31 | (250 ng/ml) Adjusted for sample dilution (20mg/ml) |

*=Average duplicate values after deducting the std zero values.

**NOTE**: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.




A typical std. assay curve (do not use this for calculating sample values)



### PRINCIPLE OF THE TEST

Bovine IgG ELISA kit is based on binding of Bovine IgG from samples to two antibodies, one immobilized on the microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of IgG present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of IgG in samples and control is read off the standard curve.

### MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000ul) and multi-channel pipette with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

### PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Sample Diluent and Antibody-HRP contain Proclin 300 (0.05%, v/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid and Proclin 300, if not already on file, can be requested or obtained from the ADI website.

### SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed , these could be stored at -20$^{O}$C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum. It is also possible to use plasma for testing.

### Reagent Preparation

1. Dilute the Sample Diluent 1:20 with water (5ml diluent in 95ml water). Dilute only the required reagent. Store diluted solution at 2-8°C for 3-4 days. Prepare 100ml for a full plate assay.

2. Dilute the wash buffer 1:100 with water. Dilute 5ml of the stock in 495ml water. Store at room temperature for 1 week.

3. Dilute the HRP conjugate1:100 with 1x sample diluent (100ul diluent in 9.9 ml diluent). Dilute only the required reagent. Prepare 10 ml for a full plate assay.

**STORAGE AND STABILITY**

The microtiter well plate and all other reagents, if unopened, are stable at 2-8$^{O}$C until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of shipping under appropriate storage conditions.

**TEST PROCEDURE** *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

1. **Do not dilute standards**. Dilute Bovine serum samples 1:80K using 1x sample diluent. Some samples may have to be diluted more or less but 1:80K should bring most normal samples to within the testing range. Due to high sample dilution, we recommend the following diluting scheme to minimize errors.

| | Sample | Diluent | Total Volume | Dilution Factor |
|---|---|---|---|---|
| Step 1 | 5 ul undiluted samples | 495 ul | 500 ul | 1:100 |
| Step 2 | 5 ul of 1:100 | 495 ul | 500 ul | 1:10000 (10K) |
| Step 3 | 10ul of 1:10k | 70ul | 80 ul | 1:80000 (80k) |

Note: It is possible to adjust the sample dilution to make 1:60K and 1:100K and testing both sample dilutions.

2. Label or mark the microtiter well strips to be used on the plate.

3. Dispense 200-300 ul of wash buffer to all wells. Let stand for 5 to 15 minutes and discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.

4. Pipet **20ul stds**. and diluted samples into appropriate wells.

5. **Note:** for ease of loading samples it is recommended that a second **uncoated** microwell plate should be used keeping diluted samples. This enables standards or samples to be transferred quickly to the ELISA plate using multichannel pipette.

6. Add **80ul** of 1x Sample diluent to all wells. Mix gently, cover the plate and incubate at room temperature for **60 minutes**.

7. Wash the wells with **3 times** with 300 ul of 1x wash buffer.



Pipette **100 ul of Ab-enzyme** conjugate into **each well. Mix gently.** Cover the plate and incubate for **30 minutes** at room temperature. **Note:** the conjugate solution must be at room temperature**.**

8. Aspirate and wash the wells **5 times** with 1x wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

9. Add **100 ul** of HRP-substrate soln. (TMB) into each well. Mix gently. Cover the plate and incubate for **15 minutes** at room temperature. Blue color develops. **Note:** TMB solution must be at room temperature.

10. Stop the reaction by adding **100 ul of stop** solution to **all wells**. Mix gently. Blue color turns yellow.

11. Measure the absorbance at **450 nm** using an ELISA reader. Color is stable for at least 30 minutes after stopping.

**NOTES:** Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 2-8$^{O}$C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each wells the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

**DILUTION OF SAMPLES**

Samples containing more than **1000 ng/ml** Bovine IgG should be further diluted and re-tested. The results obtained should be multiplied by the appropriate dilution factor.

**CALCULATION OF RESULTS**

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards and samples. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate IgG concentrations. Read off the IgG concentrations of the control and patient samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:80k then the values must be multiplied by 80,000 and results expressed as mg/ml.