PERFORMANCE CHARACTERISTICS (continued)

Sample Recovery
High and low concentrations of purified Bovine IgG were spiked into adult and neonatal serum and colostrum. Observed assay values compared to expected values ranged from 90 to 109%, indicating accurate quantification of IgG in Bovine serum and colostrum.

Sample	Expected ng/ml	Observed ng/ml	Observed/ Expected
High IgG Spike		81.8	
+ Adult Bovine Serum, 90.8 ng/ml	172	177	**102 %**
+ Neonatal Bovine Serum, 89.2 ng/ml	171	184	**108 %**
+ Bovine Colostrum, 65.8 ng/ml	160	164	**103 %**
Low IgG Spike		21.0	
+ Adult Bovine Serum, 90.8 ng/ml	118	121	**109 %**
+ Neonatal Bovine Serum, 89.2 ng/ml	110	119	**108 %**
+ Bovine Colostrum, 65.8 ng/ml	99	89	**90 %**

ELISA Kit Components	Amount	Part No.
Anti-Bovine IgG Microwell Strip Plate	8-well strips (12)	8011
Bovine IgG Control	0.65 ml	8012
Bovine IgG Standard 10 ng/ml	0.65 ml	8013B
Bovine IgG Standard 25 ng/ml	0.65 ml	8013C
Bovine IgG Standard 50 ng/ml	0.65 ml	8013D
Bovine IgG Standard 100 ng/ml	0.65 ml	8013E
Bovine IgG Standard 200 ng/ml	0.65 ml	8013F
Anti-Bovine IgG HRP Conjugate (100X)	0.15 ml	8014
Sample Diluent Concentrate (20X)	10 ml	SD-20B
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-8010

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

Instruction Manual No. M-8010

Bovine IgG ELISA Kit

Cat. No. 8010, 96 tests

For Quantitative Determination of Bovine Immunoglobulin G in Biological Fluids

For research use only (RUO), not for diagnosis, cure or prevention of the disease.

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

INTENDED USE

The Bovine IgG ELISA Kit is a sandwich ELISA for the quantification of IgG circulating in serum or in other appropriately qualified samples from tissue fluids (e.g., saliva, mucosa), or in cultures of bovine cells. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

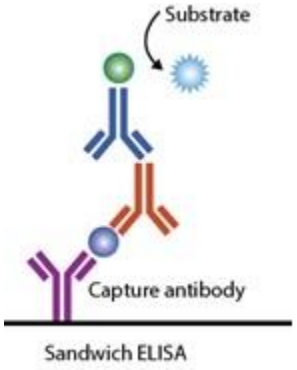
RESEARCH USE OF THE TEST

Immunoassays using heavy-chain specific antibodies provide for selective, sensitive quantification of bovine immunoglobulins IgG, IgA and IgM, as found circulating in blood or as present in other body fluids, including saliva, milk/colostrum, ascites, tears and mucosa of linings of the gut, respiratory or urigenital tracts.

Levels of total IgG, IgA and/or IgM can reveal health status or results of experimental or pathological conditions (e.g., hypo- or hypergammaglobulinemia or acute or chronic infection). Also, measurements of specific antibody levels, in antigen-specific assays, are often best interpreted relative to values of total IgG, IgA, and IgM in the sample and/or individual.

The quantitative immunoassays measure bovine IgG, IgA and IgM with high sensitivity; this allows dilution beyond interference from the sample matrix for samples derived from any of the above specimen types. Also, each assay is Ig class specific, such that all IgG or IgA subclasses are reliably quantified in essentially any specimen, freshly obtained and/or suitable stored. Expected performance of each kit relative to precision, recovery and linearity of dilution is presented as guidance for use and experimental design.

PRINCIPLE OF THE TEST



The Bovine IgG ELISA kit is based on the binding of bovine IgG in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of IgG present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of IgG in samples and control is calculated from a curve of standards containing known concentrations of IgG.

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 box label. Stabilities of the working solutions are indicated under Reagent Preparation.

PERFORMANCE CHARACTERISTICS

Specificity

The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with IgG, and have essentially no reactivity with IgM, IgA, IgE or any other bovine serum proteins.

Serum from the following species showed no significant reactivity at 1:400 dilution: mouse, rat, hamster, guinea pig, monkey, pig, horse, sheep, goat, dog, cat, rabbit or chicken.

Normal Range

Serum and colostrums IgG levels from normal animals are above 1 mg/ml. Each laboratory should determine expected values of its own testing population.

Precision

Samples containing low, medium and high concentrations of IgG were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficient of variations were calculated for the concentrations using a point-to-point curve-fitting program.

IgG concentrations were measured with good within-assay (3.0 to 4.6 %CV) and good between-assay (3.6 to 9.5 %CV) reproducibility.

Sample	IgG ng/ml	Intra-assay %CV	Inter-assay %CV
Low Sample	28.8	**3.9**	**9.5**
Medium Sample	64.1	**3.0**	**3.6**
High Sample	103.7	**4.6**	**4.5**

Linearity of Dilution

Individual samples of adult and neonatal serum, reconstituted dried colostrums (Imutek, Colorado; 40% w/v in water), and purified bovine IgG were diluted to 2 levels for testing, and concordance of the assay values were compared. The mean recovery ranged from 91 to 99%, demonstrating linear dilution and equivalent quantification across the standard range.

Sample	Dilution	Assay Value ng/ml	Serum Value mg/ml	Concordance
Adult serum	1:160k 1:1280k	181.5 27.3	29.0 34.9	**91 %**
Neonatal serum	1:50k 1:400k	178.5 24.7	8.93 9.88	**95 %**
Colostrum	1:320k 1:2560k	162 20.7	51.8 53.0	**99 %**
Pure Bovine IgG	1:5k 1:40k	163.5 21.3	0.82 0.85	**98 %**

Continued on Page 7.

CALCULATION OF RESULTS

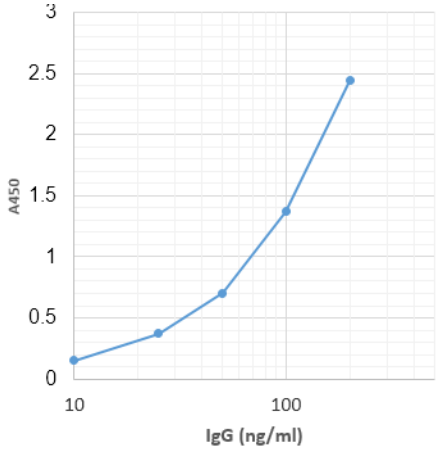
1. The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, IgG concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples.
3. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of IgG (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
4. The IgG concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
5. Multiply the values obtained for the samples by the dilution factor of each sample.
6. Samples producing signals higher than the 200 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm	IgG ng/ml
A1, A2	**Negative Diluent Control**	0.04	0
B1, B2	10 ng/ml **Standard**	0.15	10
C1, C2	25 ng/ml **Standard**	0.37	25
D1, D2	50 ng/ml **Standard**	0.77	50
E1, E2	100 ng/ml **Standard**	1.47	100
F1, F2	200 ng/ml **Standard**	2.44	200
G1, G2	**Positive Serum Control** [Value: 45 - 83 ng/ml]	1.05	66
H1, H2	**Sample** [Diluted 1:120k]	0.52	33
	Calculated: 120k-fold dilution x 33 ng/ml = **3.96** mg/ml in serum		

A typical assay Standard Curve (do not use for calculating sample values)



KIT CONTENTS

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8ºC until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at RT until kit is used entirely.
Anti-Bovine IgG - HRP Conjugate Concentrate (100x) Part No. 8014, 0.15ml	Peroxidase conjugated anti-Bovine IgG in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8ºC storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Bovine IgG Microwell Strip Plate	8011	8-well strips (12)	Coated with purified anti-Bovine IgG antibodies, and post-coated with stabilizers.
Bovine IgG Standards			
10 ng/ml 25 ng/ml 50 ng/ml 100 ng/ml 200 ng/ml	8013B 8013C 8013D 8013E 8013F	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Five (5) vials, each containing bovine serum with calibrated IgG concentrations; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
Positive Control [IgG] range on label	8012	0.65 ml	Bovine serum with stated IgG concentration range; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Bovine IgG-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera are not assayed immediately , store refrigerated for up to 2 weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.
The use of plasma has not been investigated, but should be a suitable specimen for assay.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, Sample Diluent, and Anti-Bovine IgG-HRP contain Bromo-nitro-dioxane (BND: 0.05%, w/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 BND, if not already on file, can be requested or obtained from the ADI website.

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls A Positive Serum Control is provided with the kit, assigned with an IgG concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. A Negative Diluent Control should be of lower signal than the lowest standard. Do not rely on results generated from an assay with these issues.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 100k-fold are appropriate for most normal bovine sera. For accuracy, three dilution steps are recommended, as follows:
1) 10ul serum + 390ul diluent = [1:40],
2) 20ul [1:40] + 980ul diluent = [1:2k],
3) 20ul [1:2k] + 980ul diluent = **1:100k**

DO NOT diluted the Standards or Control.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Before sample addition, add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes.
- Aspirate or dump the liquid and pat dry on a paper towel.

2. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Bovine IgG-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

5. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

6. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.