PERFORMANCE CHARACTERISTICS (continued)

Sample Recovery
High and low concentrations of purified sheep IgG were spiked into each of 3 serum samples. Observed assay values compared to expected values ranged from 96 to 130%, indicating accurate quantification of IgG in mouse serum.

Sample	Expected ng/ml	Observed ng/ml	Observed/ Expected
High IgG Spike		50.8	
+ Sheep A, 16.8 ng/ml	67.6	74.0	**109 %**
+ Sheep B, 32.0 ng/ml	82.8	82.8	**100 %**
+ Sheep C, 11.5 ng/ml	62.2	61.1	**98 %**
Low IgG Spike		19.05	
+ Sheep A, 16.8 ng/ml	35.8	34.4	**96 %**
+ Sheep B, 32.0 ng/ml	51.0	49.8	**98 %**
+ Sheep C, 11.5 ng/ml	30.5	39.6	**130 %**

Related Items
Catalog# ProdDescription

710-105-BSG Sheep Anti-Bovine Serum Albumin (BSA) IgG ELISA Kit, 96 tests,
7610-Fab Sheep/Ovine Fab ELISA Kit, 96 tests, Quantitative
7615-Fc Sheep IgG-Fc ELISA Kit, 96 tests, Quantitative
7620 Sheep IgG ELISA Kit, 96 tests, Quantitative
7630 Sheep IgM ELISA Kit, 96 tests, Quantitative
7640 Sheep IgA ELISA Kit, 96 tests, Quantitative
7650 Sheep IgE ELISA Kit, 96 tests, Quantitative

80166 Sheep Serum Antibody detection ELISA kit, Qualitative (sufficient for 500-1000 tests)

8095 Sheep Lactoferrin ELISA Kit, 96 tests, Quantitative

900-170-83G Sheep Anti-Anthrax Protective Antigen 83 (PA83) IgG ELISA kit, 96 tests, quantitative

900-260-LFG Sheep Anti-Anthrax Lethal Factor (LF) IgG ELISA kit, 96 tests
900-360-EFS Sheep Anti-Anthrax Edema Factor (EF) IgG ELISA kit, 96 tests

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

Instruction Manual No. M-7620

Sheep IgG ELISA Kit

Cat. No. 7620, 96 tests

For Quantitative Determination of Sheep
Immunoglobulin G in serum, plasma or other biological Fluids

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

ALPHA DIAGNOSTIC
INTERNATIONAL

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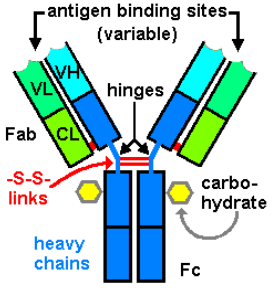
Email: service@4adi.com
www.4adi.com

INTENDED USE

The Alpha Diagnostics Int'l Sheep 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 biological solutions. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

RESEARCH USE OF THE TEST



Immunoglobulin G (IgG)

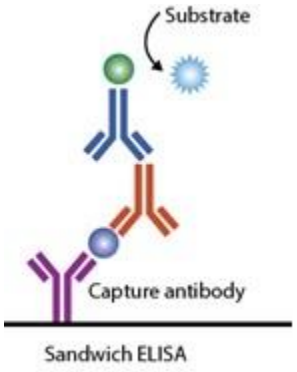
Immunoglobulin G (IgG) is a type of antibody. It is a protein complex composed of four peptide chains—two identical heavy chains and two identical light chains arranged in a Y-shape typical of antibody monomers. IgG has molecular weight of approximately 150 kDa, heavy or H chain approximately 50 kDa and light or L chain 25 kDa. Each IgG has two antigen binding sites. Representing approximately 75% of serum antibodies in humans, IgG is the most common type of antibody found in the circulation

Immunoassays using heavy-chain specific antibodies provide for selective, sensitive quantification of sheep immunoglobulins IgG, IgA and IgM, as found circulating in blood or as present in other body fluids, including saliva, milk/colostrums, ascites, tears and mucosa of linings of the gut, respiratory or urinogenital 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 the concomitant determination of total IgG, IgA, and IgM in the sample and/or individual.

The quantitative immunoassays measure sheep IgG, IgA and IgM with high sensitivity, that allows dilution beyond interference from the sample matrix for samples derived from any of the above specimen types, or from other biological solutions containing IgG. Expected performance of the assay relative to precision, linearity and normal values is presented for guidance of use.

PRINCIPLE OF THE TEST



The Sheep IgG ELISA kit is based on the binding of sheep 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 sheep IgG.

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, or any other sheep serum proteins.

IgG from bovine and goats show substantial cross-reactivity (see Caution, p1,3). Serum from the following species showed no significant reactivity at 1:400 dilution: mouse, rat, hamster, guinea pig, dog, cat, rabbit & chicken; human, monkey & pig showed some reactivity.

Normal Range

A limited testing of adult sheep sera gave values of 7.6 – 40 mg/ml . Hyper-immunized animals have elevated IgG levels; very young animals have lower levels. 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 (CVs) were calculated for the concentrations using a point-to-point curve-fitting program.

IgG concentrations were measured with very good within-assay (2.5 to 6.2 %CV) and between-assay (1.1 to 2.4 %CV) reproducibility.

Sample	IgG ng/ml	Intra-assay %CV	Inter-assay %CV
Low Sample	13.4	**2.6**	**1.1**
Mid Sample	31.5	**2.5**	**1.9**
High Sample	57.0	**6.2**	**2.4**

Linearity of Dilution

Two (2) individual sera, one (1) IgG purified by affinity-purification, and one (1) IgG prepared by standard methods (>95% pure), were diluted to 2 levels for testing, and concordance of the assay values were compared. The mean recovery ranged from 94 to 100%, demonstrating linear dilution and equivalent quantification across the standard range.

Sample	Dilution	Assay Value ng/ml	Serum Value mg/ml	Concordance
Sheep Serum #1	1:150k 1:1200k	132 15.4	19.8 18.5	**96 %**
Sheep Serum #2	1:75k 1:600k	133 14.9	9.98 8.94	**94 %**
Sheep IgG Affinity-pure	1:10k 1:80k	62.8 8.2	0.63 0.66	**98 %**
Sheep IgG >95% pure	1:12k 1:96k	82.5 10.4	0.99 1.00	**100 %**

CALCULATION OF RESULTS

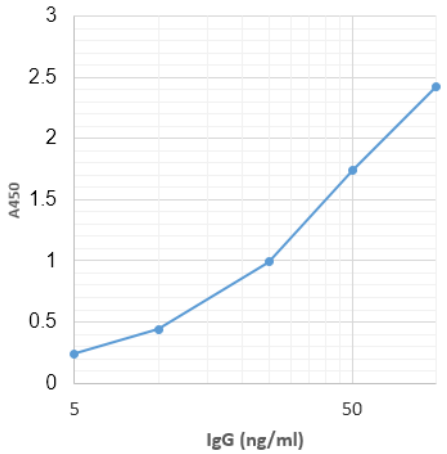
The results may be calculated using any immunoassay software package, or by plotting the data on semi-log graph paper. The four-parameter curve-fit is recommended; for hand graphing a point-to-point curve is most reliable.
The sheep IgG concentrations in unknown samples and controls can be determined by interpolation from the standard curve, and then multiplication of the values by the dilution factor to obtain IgG concentration in the original prep. Samples producing signals higher than the 100 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	5 ng/ml **Standard**	0.24	5
C1, C2	10 ng/ml **Standard**	0.44	10
D1, D2	25 ng/ml **Standard**	0.99	25
E1, E2	50 ng/ml **Standard**	1.74	50
F1, F2	100 ng/ml **Standard**	2.42	100
G1, G2	**Positive Serum Control** [Value: 49 - 91 ng/ml]	1.25	32
H1, H2	**Sample** [Diluted 1:600k]	0.69	15
	Calculated: 600k-fold dilution x 15 ng/ml = **9.0** mg/ml in serum		

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



/B3/7620-ELISA-Graph

KIT CONTENTS

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20B, 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-Sheep IgG - HRP Conjugate Concentrate (100x) Part No. 7624, 0.15ml	Peroxidase conjugated anti-Sheep IgG in buffer with detergents and ProClin 300 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 2-8º C storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Sheep IgG Microwell Strip Plate	7621	8-well strips (12)	Coated with purified anti-sheep IgG antibodies.
Sheep IgG Standards			
5 ng/ml 10 ng/ml 25 ng/ml 50 ng/ml 100 ng/ml	7623B 7623C 7623D 7623E 7623F	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Five (5) vials, each containing sheep IgG of designated concentrations; diluted in buffer with detergents and ProClin 300 as stabilizers.
Positive Control [IgG] range on label	7622	0.65 ml	Sheep IgG of stated concentration range; diluted in buffer with detergents and ProClin 300 as stabilizers.
TMB Solution	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

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

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 Antibody-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.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, 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

Caution! The Sheep IgG antibodies significantly cross-react with IgG from bovine and sheep. The BSA used in many diluents, including bovine serum in culture media, often contains bovine IgG as a minor component, which will produce (background) signals in this assay when used in a sample. It is best to avoid BSA in samples.

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference.

For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature.

For **other samples**, including bovine serum-free tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately , store refrigerated for up to a week, or frozen for long-term storage. Avoid freeze-thaw cycles.

ASSAY PROCEDURE

Bring all reagents to lab temperature (18-30^{o} C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 500k-fold are appropriate for most normal sheep sera. For accuracy, three dilution steps are recommended, as follows:

1) 10ul serum + 990ul diluent = [1:100],
2) 10ul [1:100] + 990ul diluent = [1:10k],
3) 10ul [1:10k] + 490ul diluent = **[1:500k].**

DO NOT dilute the Standards or Positive Control Serum.

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.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes before sample addition.
- Aspirate 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-Sheep 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.