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.

Technique Accurate and reproducible assay results rely on good lab technique regarding pipetting, plate washing and handling of samples and reagents.

Related Items

Catalog#	Description
7520	Goat IgG ELISA Kit, 96 tests, Quantitative
7520-RDT-25	TruStrip RDT Goat IgG Rapid Test cards, 10/pk
7530	Goat IgM ELISA Kit, 96 tests, Quantitative
7540	Goat IgA ELISA Kit, 96 tests, Quantitative
7550	Goat IgE ELISA Kit, 96 tests, Quantitative
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
7620-RDT-25	TruStrip RDT Sheep IgG Rapid Test cards, 10/pk
7630	Sheep IgM ELISA Kit, 96 tests, Quantitative
7640	Sheep IgA ELISA Kit, 96 tests, Quantitative
7650	Sheep IgE ELISA Kit, 96 tests, Quantitative

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

Instruction Manual No. M-7520

Goat IgG ELISA Kit

Cat. No. 7520, 96 Tests

For Quantitative Determination of Goat Immunoglobulin G in Biological Fluids

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

ALPHA DIAGNOSTIC INTERNATIONAL

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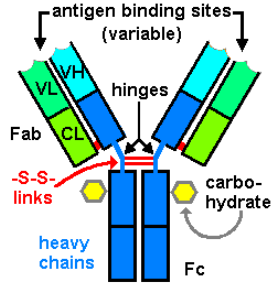
www.4adi.com

INTENDED USE

The Alpha Diagnostics Int'l Goat 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.

RESEARCH USE OF THE TEST



Immunoglobulin G (IgG)

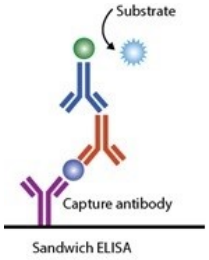
Immunoglobulin G (IgG) is a type of antibody. It is a protein complex composed of four peptide chains—two identical heavy chains and two identical light chains arranged in a Y-shape typical of antibody monomers. IgG has molecular weight of approximately 150 kDa, heavy or H chain approximately 50 kDa 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 goat 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 urogenital 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 goat 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 Goat IgG ELISA kit is based on the binding of goat 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 goat IgG.

Caution! The Goat 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.

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 goat serum proteins.

IgG from bovine and sheep show substantial cross-reactivity. Avoid sample diluents with BSA which may contain IgG, or culture medium containing bovine IgG, as signals due to these additives will interfere with accurate quantitation of goat IgG in the sample.

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

Normal Range

A limited testing of adult goat sera gave values of 7.2 – 43 mg/ml . Hyper-immunized animals have elevated IgG levels; young animals will be lower. 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 good within-assay (4.1 to 7.0 %CV) and between-assay (5.4 to 13.7 %CV) reproducibility.

Sample	IgG ng/ml	Intra-assay %CV	Inter-assay %CV
Low Sample	50	**5.2**	**7.6**
Mid Sample	148	**4.1**	**5.4**
High Sample	311	**7.0**	**13.7**

Linearity of Dilution

Three (3) individual sera, and one (1) purified IgG preparation, were diluted to 2 levels for testing, and concordance of the assay values were compared. The mean recovery ranged from 90 to 99%, demonstrating linear dilution and equivalent quantification across the standard range.

Sample	Dilution	Assay Value ng/ml	Serum Value mg/ml	Concordance
Goat Serum #1	1:30k 1:240k	427 59.9	12.8 14.4	**94 %**
Goat Serum #2	1:100k 1:800k	449 68.9	44.9 55.1	**90 %**
Goat Serum #3	1:100k 1:800k	334 41.1	33.4 32.9	**99 %**
Goat IgG	1:40k 1:160k	146.7 40.8	5.88 6.53	**95 %**

CALCULATION OF RESULTS

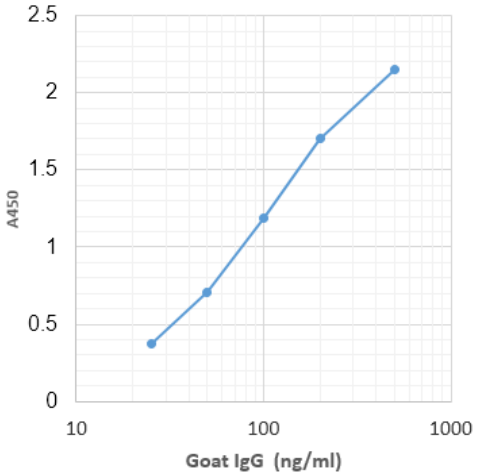
The results may be calculated using any immunoassay software package, or by plotting the data on semi-log graph paper. The four-parameter curve-fit is recommended; for hand graphing a point-to-point curve is most reliable.
The goat IgG concentrations in unknown samples and controls can be determined 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 500 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**	0.08	0
B1, B2	25 ng/ml **Standard**	0.37	25
C1, C2	50 ng/ml **Standard**	0.71	50
D1, D2	100 ng/ml **Standard**	1.19	100
E1, E2	200 ng/ml **Standard**	1.70	200
F1, F2	500 ng/ml **Standard**	2.15	500
G1, G2	**Positive Serum Control** [Value: 105 - 195 ng/ml]	1.48	157
H1, H2	**Sample** [Diluted 1:100k]	1.41	143
	Calculated: 100k-fold dilution x 143 ng/ml = **14.3** mg/ml in serum		

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



b/4-7520-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-Goat IgG - HRP Conjugate Concentrate (100x) Part No. 7524, 0.15ml	Peroxidase conjugated anti-Goat IgG in buffer with protein, detergents and antimicrobials 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-Goat IgG Microwell Strip Plate	7521	8-well strips (12)	Coated with purified anti-goat IgG antibodies.
Goat IgG Standards			
25 ng/ml 50 ng/ml 100 ng/ml 200 ng/ml 500 ng/ml	7523B 7523C 7523D 7523E 7523F	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Five (5) vials, each containing goat IgG of designated concentrations; diluted in buffer with detergents and antimicrobials as stabilizers.
Positive Control [IgG] range on label	7522	0.65 ml	Goat IgG of stated concentration range; diluted in buffer with detergents and antimicrobials as stabilizers.
TMB Solution	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 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

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute 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 can be requested or obtained from the ADI website: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf

SPECIMEN COLLECTION AND HANDLING

Caution! The Goat 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° C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 20 to 200k-fold are appropriate for most normal goat sera. For accuracy, two dilution steps are recommended, as follows:

1) 10ul serum + 990ul diluent = [1:100],
2) 5ul [1:100] + 995ul diluent = **[1:20k].**

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-Goat 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.