

ELISA kits available from ADI:

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, estradiol, testosterone, progesterone).

Monkey: IgM, IgG, IgA, IgE

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

Autoimmune Antibody detection kits for ANA, ssDNA, dsDNA, Histone, Sm, RNP, SSA, SSB, Scl70, Ovalbumin, Cardiolipin, CIC

Chicken: IgG, IgM, IgY, Ovalbumin

Turkey: IgG

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Pig: Albumin, IgG, IgM

Dog: CRP, IgG, IgM

Cat: IgG, IgM

Goat: IgG

Rabbit: CRP, IgG

Sheep: IgG

See Details at the web site or Contact ADI

Instruction Manual No. M-7420

Guinea Pig Immunoglobulin G (IgG)

ELISA KIT #. 7420

For Quantitative Determination of G. Pig IgG
In Serum, plasma or other biological fluids



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G. Pig IgG ELISA KIT Cat. No. 7420

Kit Components, 96 tests	Cat #
Anti-G. Pig IgG coated strip plate (8 wells x 12 strips)	7421
G. Pig IgG Reference Standard , lyophilized in pink buffer. Reconstitute with the specified volume of distilled water and then prepare 100 ng/ml stock (see lot specific conc on the vial)	7422
Anti-G. Pig IgG-HRP Conjugate , 11 ml	7423
Sample Diluent (10X) , 25 ml	7420-SD
Wash Buffer (20X) , 50 ml	7420-WB
TMB Substrate , 11 ml	7420-TM
Stop solution , 11 ml	7420-SS
Instruction Manual	M-7420

INTRODUCTION

Immunoassays using heavy-chain specific antibodies provide for selective, sensitive quantification of monkey 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 values of total IgG, IgA, and IgM in the sample and/or individual.

The quantitative immunoassays measure 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, IgM and 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 for guidance of use and experimental design.

ADI's G. Pig IgG ELISA provides is a very specific and sensitive assay for G. Pig IgG in serum, plasma or other biological fluids.

PERFORMANCE CHARACTERISTICS

Detection Limit: Based on 6 replicate determinations of the zero standards, the minimum IgG concentration detectable using this assay is ~2.0 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Expected Values: A limited testing of G. pig sera produced values of 5 mg/ml (range 3.5-6.5 mg/ml).

Specificity

Antibodies used in the kit are specific for G. pig IgG with no reactivity with the IgM or IgA or IgE or other serum proteins. .

Species Crossreactivity

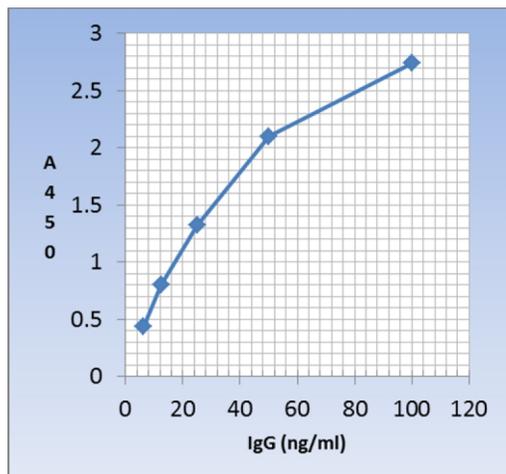
Cross-reaction of other species IgG (e.g., mouse, rat, human etc) has not been studied.

ADI provides IgG ELISA kits For Human, Mouse, Rat, Monkey, Rabbit and Dog.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A _{450 nm}	Net mean A ₄₅₀
A1, A2	Sample Diluent	0.10	
B1, B2	Standard A 3.12 ng/ml	0.37	0.27
C1, C2	Standard B 6.25 ng/ml	0.55	0.44
D1, D2	Standard C 12.5 ng/ml	0.9	0.8
E1, E2	Standard D 25 ng/ml	1.42	1.32
F1, F2	Standard E 50 ng/ml	2.2	2.1
F1, F2	Standard F 100 ng/ml	2.85	2.74
G1, G2	Sample 1	1.53	1.32

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.



17_ADL_ELISA

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

PRINCIPLE OF THE TEST

G. Pig IgG ELISA kit is based on binding of 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 (5-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS AND SAFETY INSTRUCTIONS

This ELISA Kit is for research use only.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H₂SO₄ (stop solution), and Proclin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

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, store frozen for up to six months. Avoid repeated freezing and thawing of samples. It is also possible to use plasma for testing.

REAGENT PREPARATION

1. Preparation of the Standards. Stock std vial is supplied lyophilized and upon reconstitution with the specified volume of 1x sample diluent will prepare the 100 ng/ml stock. Prepare additional standards of 50, 25, 12.5, 6.25 and 3.13 ng/ml by 2-fold serial dilution (dilute 100 ng/ml stock 1:2 or 250 ul stock and 250 ul 1x sample diluent and continue with the remaining standards. Stock vial can be stored at 4oC for 1 week or frozen at -20oC or below in suitable size aliquots.
2. The Wash Buffer is a 20x stock. Dilute the entire 50 ml with distilled or deionized water to 1 L total volume. Store at room temperature for the entire use of the kit.
3. Sample Diluent is 10x. **Dilute 1:10** with water (1 ml stock in 9 ml water). Store 1x sample diluent at 4oC..

Sample Dilution

In our limited testing, G. Pig Pig in adult normal sera samples is about 5 mg/ml. Therefore, it will be necessary to dilute the sample at least 1:100,000 before testing. We recommend perform sample dilution using the following scheme:

1. Prepare 100 dilution first or 5 ul sample into 495 ul diluent.
2. take 5 ul of 1:100 dilution and 495 ul diluent (final dilution, 1:10,000).
3. Take 25 ul of 1:10,000 into 225 ul diluent (final dilution 1:100,000)

Store 1:100 and 1:10,000 sample stocks at 4oC until analyses is complete. It is possible to perform other sample dilutions, if necessary, from the 1:10,000 stock.

Due to high dilution of the samples, it is recommended to perform the sample dilution using above scheme to minimize sample dilution errors.

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

1. Reconstitute lyophilized Reference Standard with 1 ml of distilled water and the prepare the std F using instruction on the vial and label this as stock F (100 ng/ml).. **Note: Stock concn is lot specific and the reconstitution volume is provided for each lot).** Store diluted stock conc at 4oC for 1-week or store frozen at 2o0-C for 6 months. .
2. Prepare additional liquid standards using the 2-fold serial dilution of 100 ng/ml stock scheme:

G. Pig IgG Stock		Diluent	Final Concn	Final Volume
Concn	Volume			
F.....Stock 100 ng/ml				
Stock F	250 uL	+ 250 uL	E 50 ng/ml	500 uL
Stock E	250 uL	+ 250 uL	E 25 ng/ml	500 uL
Stock D	250 uL	+ 250 uL	D 12.5 ng/ml	500 uL
Stock C	250 uL	+ 250 uL	C 6.25 ng/ml	500 uL
Stock B	250 uL	+ 250 uL	B 3.12 ng/ml	500 uL

3. Label or mark the microtiter well strips to be used on the plate.
4. Pipet **100 ul** standards and diluted samples in duplicate into appropriate wells.

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.

5. Mix gently, and incubate on at orbital micro-plate shaker at 150 rpm at room temp.. (18-25 oC) for **45 minutes**
6. Wash the wells **4 times with 300 ul** of 1x wash buffer.

7. Pipette **100 ul** of Ab-enzyme conjugate into each well. Mix gently, and incubate on at orbital micro-plate shaker at 150 rpm for **45 minutes** 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. Remove the traces of wash buffer by adsorbing the plate over a clean paper towels.
9. Add **100 ul of TMB** Substrate into each well. Mix gently. Cover the plate and incubate on plate shaker at 150 rpm for **20 minutes** at room temperature. Blue color develops.
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°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 100 ng/ml IgG should be further diluted and re-tested. The results obtained should be multiplied by the appropriate dilution factor. It is possible to use normal saline or PBS for sample dilution if larger volumes of samples are taken for dilution or if more sample diluent is required.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on a 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 1:100,000 then the values must be multiplied by 100,000.