ELISA kits available from ADI:

Catalog#	ProdDescription
9020	Pig IgG ELISA Kit, 96 tests, Quantitative (swine/porcine)
9080	Pig IgM ELISA Kit, 96 tests, Quantitative
9000	Pig Albumin ELISA Kit, 96 tests, Quantitative
1780	Human IgA ELISA Kit, 96 tests, Quantitative
6310	Mouse IgA ELISA Kit, 96 tests, Quantitative
6440	Rat IgA ELISA Kit, 96 tests, Quantitative
6510	Rabbit IgA ELISA Kit, 96 tests, Quantitative
7010	Monkey IgA ELISA Kit, 96 tests, Quantitative
7720	Horse IgA ELISA Kit, 96 tests, Quantitative
8075	Bovine IgA ELISA Kit, 96 tests, Quantitative
600-610-HMY	Human Myoglobin ELISA Kit
600-620-MMY	Monkey Myoglobin ELISA Kit
600-630-MMY	Mouse Myoglobin ELISA Kit
600-650-RMY	Rabbit Myoglobin ELISA Kit
600-660-RMY	Rat Myoglobin ELISA Kit

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, Troponin-I, 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 **Sheep:** IgG **Goat:** IgG **Rabbit**: CRP, IgG
See Details at the web site or Contact ADI

Instruction Manual No. M-9010

Pig IgA

ELISA KIT Cat. # 9010, 96 Tests

For quantification of IgA class antibodies in Pig Serum or Plasma

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

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Pig IgA ELISA KIT # 9010

Kit Components, 96 tests	Cat #
Anti-pig IgA coated strip plate (8 wells x 12 strips), #90101	1 plate
Pig IgA **Reference Standard, Lyophilized**, Store at -20oC , #90102	1 vial
HRP Conjugate, 11 ml, #90103	1 bottle
Sample Diluent (10X), 25 ml, #90104	1 bottle
Wash Buffer (20X), 50 ml, #9010-WB	1 bottle
TMB Substrate, 11 ml, #9010-TMB	1 bottle
Stop solution, 11 ml, #9010-SS	1 bottle
Instruction Manual, # M-9010	1

Intended Use

ADI's Pig IgA ELISA kit is a highly sensitive sandwich type assay for the quantitative measurement of IgA class antibodies in pig serum or plasma or cell culture supernatant or other biological samples and. This kit is for in vitro research use only (RUO). ADI also has kits for detection of IgA class antibodies in human, rat, mouse, monkey and other species.

INTRODUCTION

Immunoglobulin A (IgA, also referred to as sIgA) is an antibody that plays a critical role in mucosal immunity. More IgA is produced in mucosal linings than all other types of antibody combined; between three and five grams are secreted into the intestinal lumen each day. This accumulates up to 15% of the total immunoglobulin produced in the entire body. IgA has two subclasses (IgA1 and IgA2) and can exist in a dimeric form called secretory IgA (sIgA). While IgA1 predominates in serum (~80%), IgA2 percentages are higher in secretions than in serum (~35% in secretions);[6] the ratio of IgA1 and IgA2 secreting cells varies in the different lymphoid tissues of the human body. In its secretory form, IgA is the main immunoglobulin found in mucous secretions, including tears, saliva, sweat, colostrum and secretions from the genitourinary tract, gastrointestinal tract, prostate and respiratory epithelium. It is also found in small amounts in blood. The secretory component of sIgA protects the immunoglobulin from being degraded by proteolytic enzymes, thus sIgA can survive in the harsh gastrointestinal tract environment and provide protection against microbes that multiply in body secretions. sIgA can also inhibit inflammatory effects of other immunoglobulins. IgA is a poor activator of the complement system, and opsonises only weakly. Its heavy chains are of the type α.

The ratio of IgA1 and IgA2 secreting cells varies in the different lymphoid tissues of the human body: IgA1 is the predominant IgA subclass found in serum. Most lymphoid tissues have a predominance of IgA-producing cells. In IgA2, the heavy and light chains are not linked with disulfide, but with noncovalent bonds. In secretory lymphoid tissues (e.g., gut-associated lymphoid tissue, or GALT), the share of IgA2 production is larger than in the non-secretory lymphoid organs (e.g. spleen, peripheral lymph nodes). Both IgA1 and IgA2 have been found in external secretions like colostrum, maternal milk, tears and saliva, where IgA2 is more prominent than in the blood. Polysaccharide antigens tend to induce more IgA2 than protein antigens.

4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of IgA in the sample.

5. Ideally, PC graphing software should be used for the above steps. We find good fits of standard data to a second order polynomial equation

6. If the OD450 values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

Quality Control

Full set of reference standards must be run with each run. Reference standard should closely reflect the values shown in this manual. Blanks must be less than A450=0.300. Higher blanks is an indication of poor washing. Repeat the stds only with proper washing to confirm the expected values.

PERFORMANCE CHARACTERISTICS

Wash Procedure: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Detection Limit: The minimum IgA concentration detectable using this assay is **2.5 ng/ml**. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Expected Values: Each laboratory should establish testing ranges for the animal population being investigated.

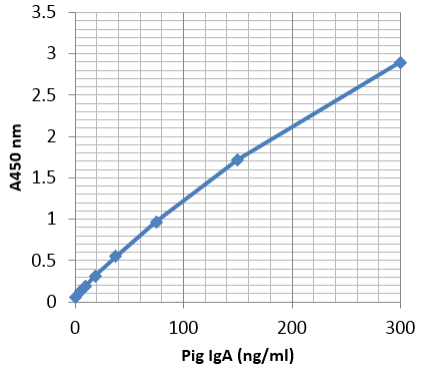
Species reactivity: The antibodies in this kit react with min-pig as well. Other species not tested. ADI has species specific IgA, IgG, and IgM kits This kit is optimized for Pig IgA. IgA detection of other species has not been established. Separate kits are available for detection in other species.

References: http://en.wikipedia.org/wiki/Immunoglobulin_A; S Fagarasan and T Honjo (2003). "Intestinal IgA Synthesis: Regulation of Front-line Body Defenses". Nature Reviews Immunology 3 (1): 63–72; Holmgren, J; Czerkinsky, C (April 2005). "Mucosal immunity and vaccines". Nature Medicine 11 (4s): S45–S53; Snoeck V, Peters I, Cox E (2006). "The IgA system: a comparison of structure and function in different species". Vet. Res. 37 (3): 455–67. AJ Macpherson and E Slack. (2007). "The functional interactions of commensal bacteria with intestinal secretory IgA.". Current Opinion in Gastroenterology 23 (6): 673–678

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples		Mean A_{450} nm	Calculated Concn
A1, A2	**Diluent**	0 ng/ml	0.057	
B1, B2	**Standard A**	4.69 ng/ml	0.122	
C1, C2	**Standard B**	9.38 ng/ml	0.184	
D1, D2	**Standard C**	18.75 ng/ml	0.314	
E1, E2	**Standard D**	37.5 ng/ml	0.552	
F1, F2	**Standard E**	75 ng/ml	1.067	
G1, G2	**Standard F**	150 ng/ml	1.719	
H1, H2	**Standard G**	300 ng/ml	2.897	
A3, A4	**Sample 1**			ng/ml

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.



ADI_ELISA4

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

CALCULATION OF RESULTS:

1. 1.Calculate the average absorbance values (A450) for blanks and each set of reference standards and samples. Deduct the average blank values from the stds and samples (net values).

2. Construct a standard curve by plotting the net mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.

3. Using the mean absorbance value for each sample, determine the corresponding concentration of IgA in ng/ml from the standard curve.

4.

It is also possible to distinguish forms of IgA based upon their location - serum IgA vs. secretory IgA. In secretory IgA, the form found in secretions, polymers of 2-4 IgA monomers are linked by two additional chains; as such, the molecular weight of sIgA is 385,000D. One of these is the J chain (joining chain), which is a polypeptide of molecular mass 15kD, rich with cysteine and structurally completely different from other immunoglobulin chains. This chain is formed in the IgA-secreting cells. The oligomeric forms of IgA in the external (mucosal) secretions also contain a polypeptide of a much larger molecular mass (70 kD) called the secretory component that is produced by epithelial cells. This molecule originates from the poly-Ig receptor (130 kD) that is responsible for the uptake and transcellular transport of oligomeric (but not monomeric) IgA across the epithelial cells and into secretions such as tears, saliva, sweat and gut fluid.

Levels of total IgG, IgA and/or IgM can reveal health status or results of experimental or pathological conditions (e.g., hypo- or hyper gammaglobulinemia 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 IgG in the sample and/or individual.

PRINCIPLE OF THE TEST

Pig IgA ELISA kit is based on binding of Pig IgA 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 PMY 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 PMY 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. Plate shaker or orbital shaker; Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS AND SAFETY INSTRUCTIONS

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 Prolcin-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 cannot be immediately assayed, store frozen for up to six months. Avoid repeated freezing and thawing of samples. **Cell or tissues extract samples have not been optimized.**

Sample Dilution

IgA concentration in serum or plasma is typically 1 mg/ml. therefore, sample must be diluted 1:20,000 to bring them within testing range. It is a good idea to run a few samples at 1:20,000 and 1:40,000 to make sure that they are falling with the testing range.

1. Dilute 5 ul sample with 495 ul 1x sample diluent (dilution 1:100)
2. Take 2.5 ul of 1:100 stock sample in 497.5 ul of diluent (total dilution 1:20,000).
3. It is possible to vary the dilution at step to make 1:5,000-10,000 for low IgA values) or prepare additional dilution (e.g. 1:40,000 for samples with high IgA values).

REAGENT PREPARATION

1. **Dilute Wash Buffer (20x stock)**. Dilute the entire 50 ml with 950 ml of distilled or deionized water (total volume 1000 ml). Store at room temperature for the entire use of the kit. It can be stored at 4oC for long term storage.

2. **Sample Diluent** is 10X. **Dilute 1:10** with water (1 ml stock in 9 ml water). Store 1x sample diluent at 4oC..

3. **Reference Standard** is provided as lyophilized power (pink). Dilute it with 1x sample diluent to make standards as given below.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8oC until the expiration date printed on the label. The Pig IgA reference standard should be stored at -20oC.

Preparation of Standards

1. The concentration of the reference lyophilized standard (Pink colored powder; ug/ml) is provided on the vial ug/ml. This is reconstituted in 1 ml water to prepare stock solution and further diluted to **prepare 300 ng/ml standard** (**Std G**) is also given on the vial. Stock standards are stable for up to 24-hrs at 4oC. Prepare other refs. standards (150-4.69 ng/ml by 2-fold serial dilution) fresh prior to the assay and do not store for more than 1-2 hr. Immediately aliquot and store any unused stock reference standard at –20oC or below.

2. Prepare the remaining standards (F-A) as shown below. You will need 200 ul of each standard to run as duplicate on 100 ul/wells.

Pig IgA Stds	Stock Volume	1X Sample. diluent	Final Volume
Std G (300 ng/ml) Prepared in step 1	500 uL	0	500 uL
Std F (150 ng/ml)	250 uL of Std G	250 uL	500 uL
Std E (75 ng/ml)	250 uL of Std F	250 uL	500 uL
Std D (37.5 ng/ml)	250 uL of Std E	250 uL	500 uL
Std C (18.75 ng/ml)	250 uL of Std D	250 uL	500 uL
Std B (9.38 ng/ml)	250 uL of Std C	250 uL	500 uL
Std A (4.69 ng/ml)	250 uL of Std B	250 uL	500 uL
Diluent (0 ng/ml)	0	250 uL	250 uL

Notes: When preparing the serial dilutions of the standards gently mix the standards for 5-10 seconds and then take aliquots to make further dilutions. Following the above dilution scheme, you will have 100 uL of negative and all standards (B-F), 900 uL of std. G and 200 ul of Std. A. You would need 40 uL of each standard (20 uL in duplicate).

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

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

3. Use first 2 wells for blanks (100 ul of 1x sample diluent). Pipet **100 ul standards and samples** in duplicate into appropriate wells. Mix gently, and incubate at room temperature (25oC) for **45 minutes on an orbital shaker (100-150 rpm).** If an automated shaker is not available, the plate can be mixed manually every few minutes.

4. Remove or aspirate the plate contents and **wash the wells 5 times** with 400 ul of 1x wash buffer using an automated washer. If washing manually then dump the plate contents and tap over paper towels, add wash buffer, shake the contents of 5-10 seconds and repeat the steps. Tap the plate over fresh paper towels between each washing.

5. Pipette **100 ul of HRP conjugate** into each well, and incubate at room temperature (25oC) for **45 minutes on an orbital shaker (100-150 rpm).**

6. Remove or aspirate the plate contents and **wash the wells 5-6 times** with 400 ul of 1x wash buffer as above in step 5.

7. **Add 100 ul of TMB Substrate** into each well. Mix gently. Cover the plate and incubate for **20 minutes** at room temperature **on an orbital shaker (100-150 rpm). Blue color develops in standards and positive wells**. This step can be reduced or increased by ± 5 minutes to keep the color within reading range. If your ELISA reader cannot read above A450 of 2.00-3.00 then reduce the incubation time.

8. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 30 seconds. **Blue color turns yellow.**

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

Please Note: Due to plate reader differences, the high standard absorbance values may be out of range occasionally. If this occurs, absorbance values may be determined at 405 nm instead. If absorbance values exceed the high standard, the samples should be appropriately diluted and redetermined. Samples with absorbance values below those of the lowest standard should be assigned a zero IgA value.