ELISA kits available from ADI:

Catalog#	Product Description
6240	Mouse Serum Amyloid A ELISA Kit
6250	Mouse Serum Haptoglobin ELISA Kit
6250-10	Dog Serum Haptoglobin ELISA Kit
6250-20	Horse Serum Haptoglobin ELISA Kit
6250-30	Rat Serum Haptoglobin ELISA Kit
6250-50	Cat Serum Haptoglobin ELISA Kit
6250-60	Bovine Serum Haptoglobin ELISA Kit
600-480-CTN	Rabbit Cardiac Tn-I ELISA kit for serum samples
600-510-MTN	Rat Skeletal Muscle Troponin 1 (Tn-I) ELISA Kit
600-600-DMY	Dog Myoglobin ELISA Kit
600-610-HMY	Human Myoglobin ELISA Kit
600-620-MMY	Monkey Myoglobin ELISA Kit
600-630-MMY	Mouse Myoglobin ELISA Kit
600-640-PMY	Pig Myoglobin ELISA Kit
600-650-RMY	Rabbit 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-300-180-CGA

Cat IgA ELISA KIT

Cat. No. 300-180-CGA, 96 tests

For measuring IgA in serum, plasma or other biological fluids of Cats

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

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

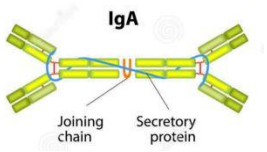
Cat IgA ELISA KIT Cat. No. 300-180-CGA

Kit Components, 96 tests	
Anti-Cat IgA **coated strip** plate (8 wells x 12 strips), #300-181P	1 plate
Cat IgA **Calibrator**, **lyophilized**, *Reconstitute with **1 ml** **dH_20** according to vial label, #300-182*	1 vial
Anti-Cat IgA-**HRP Conjugate(100X)**, **150 ul,** #300-183	1 bottle
Wash Buffer (20X), 50 ml, #300-180-WB	1 bottle
Sample Diluent Concentrate, (5X) 50 ml, #300-180-SD	1 bottle
TMB Substrate, 12 ml, #300-180-TMB	1 bottle
Stop solution, 12 ml, #300-180-SS	1 bottle
Instruction Manual, #M-300-180-CGA	1 manual

Intended use:

ADI's Cat IgA ELISA provides is a rapid, specific and sensitive assay for measuring
IgA in cat serum, plasma or other biological solutions. Research Use Only (RUO), not for therapeutic use.

INTRODUCTION:



IgA is the predominant immunoglobulin class in body secretions, such as saliva, tears, bronchial secretions, nasal mucosal secretions, prostatic fluid, vaginal secretions, and mucous secretions of the small intestines. It may serve both to defend against local infection and to prevent access of foreign antigens to the general immunologic system. It is also found in small amounts in blood. Because it is resistant to degradation by enzymes, secretory IgA can survive in harsh environments such as the digestive and respiratory tracts, to provide protection against microbes that multiply in body secretions. IgA does not activate complement, and opsonises only weakly. Its heavy chains are of the type α. It exists in two forms, IgA1 (90%) and IgA2 (10%): IgA1 is found in serum and made by bone marrow B cells. In IgA2, the heavy and light chains are not linked with disulfide but with noncovalent bonds. IgA2 is made by B cells located in the mucosae and has been found to secrete into colostrum, maternal milk, tears and saliva.
IgA is found in secretion in a specific form called secretory IgA, a dimer of two IgA monomers linked by two additional chains: One of these is the J chain (from join), which is a polypeptide of molecular mass 1,5 kD, rich with cysteine and structurally completely different from other immunoglobulin chains. This chain is formed in the antibody-secreting cells. The dimeric form of IgA in the outer secretions also has a polypeptide of the same molecular mass (1,5 kD) called the secretory chain and is produced by epithelial cells. It is also possible to find trimeric and even tetrameric IgA

CALCULATION OF RESULTS

Subtract the average background value from the test values for each sample. Using the results observed for the standards construct a Standard Curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.

Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the cat IgA concentration in original samples.

PERFORMANCE CHARACTERISTICS

Intra-Assay CV < 10%

Inter-Assay CV < 10%

Control Serum Recovery > 85%

Expected Values

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

Specificity

The antibodies used in this kit are specific for cat IgA. No significant reactivity with IgG, IgM or IgE or other serum proteins.

Species Crossreactivity:

Cross-reactivity with other species is not tested. ADI has IgA ELISA kits for mouse, rat, dog, horse, pig, cat, bovine, and monkey.

STORAGE AND STABILITY

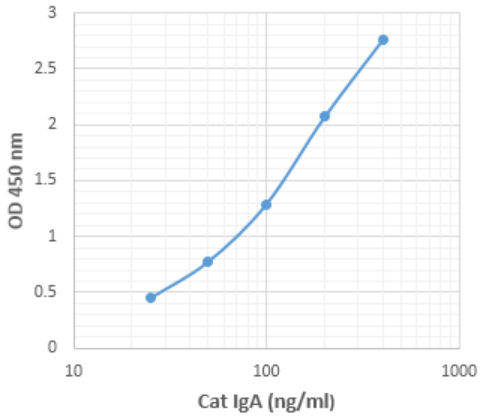
The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{O}C until the expiration date printed on the label. After opening the kit components, the shelf life is approximately 2 months.

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^{o}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 well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples		Mean $A_{450\ nm}$	Calculated Concn
A1, A2	**Standard A**	0 ng/ml	0.1	-
B1, B2	**Standard B**	12.5 ng/ml	0.28	0.180
C1, C2	**Standard C**	25.5 ng/ml	0.448	0.348
D1, D2	**Standard D**	50 ng/ml	0.776	0.676
E1, E2	**Standard E**	100 ng/ml	1.29	1.194
F1, F2	**Standard F**	200 ng/ml	2.07	1.971
G1, G2	**Standard G**	400 ng/ml	2.76	2.60
H1, H2	**Sample 1**		1.54	1.44

NOTE: These data are for demonstration purpose only. Actual values may vary slightly from above. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



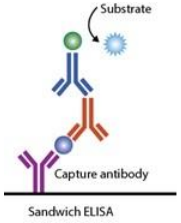
9_ADI_Elisa

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

Immunoassays using heavy-chain specific antibodies provide for selective, sensitive quantification of human 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 IgG in the sample and/or individual. The quantitative immunoassays measure human IgG, IgA and IgM with high sensitivity; this allows for sufficient dilution of the sample to avoid sample matrix interference that may occur with 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



Cat IgA ELISA kit, a sandwich ELISA, is based on binding of 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 IgA 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 IgA 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

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

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. **Sample diluent concn. (5X)** Dilute 1:5 (5 ml stock in 20 ml distilled water). Dilute only the required reagent. Store diluted solution at 2-8º C.

2. **Wash Buffer (20X stock)**. Dilute the entire **50 ml with distilled or deionized water to 950 ml water** (total volume 1000 ml). Store at room temperature for the entire use of the kit.

3. ENZYME-ANTIBODY CONJUGATE (100X)

Prepare 1X conjugate by diluting 100x stock (10 ul stock in 1 ml of 1X sample diluent). Prepare 1 ml for each strip or 10 ml for entire plate. Prepare 1x stock as necessary and do not store 1X stock after the assay.

Preparation of Standards

Reconstitute the lyophilized calibrator with 1 ml of distilled or deionized water to to prepare **stock std H** of 100 ug/ml cat IgA. Mix gently by rocking for 5-10 mins at room temp. Do not vortex to create foam. Immediately aliquot (100 ul) and store any unused reference standard at -20oC or below. Use fresh 100 ug/ml vial to prepare working standards as follows.

Stds. #	Std. Volume	Volume of 1x Diluent	Total Volume	Final Std Concn (ng/ml)
G	5 ul of Std H	1245 ul	1245 ul	400
F	250 ul of stand. G	250 ul	500 ul	200
E	250 ul of stand. F	250 ul	500 ul	100
D	250 ul of stand. E	250 ul	500 ul	50
C	250 ul of stand. D	250 ul	500 ul	25
B	250 ul of stand. C	250 ul	500 ul	12.5
A			500 ul	0

DILUTION OF SAMPLES

The assay for quantification of cat IgA requires that each test sample be diluted before use. An approximate dilution 1:20,000 of serum or plasma may be appropriate for most samples. Some samples may require different dilution to bring it within the detection range of the kit. **If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.**

Sample (serum/plasma_	1X sample diluent	Volume	Dilution
5 ul	495 ul	500 ul	1:100
5 ul of 1:100	995 ul	500 ul	1:20,000

Mix thoroughly at each stage. Store diluted samples at 2-8oC for testing or freeze at -20oC. It is better to use freshly diluted samples for testing.

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

1. Reconstitute the lyophilized calibrator and prepare working standards as outlined on page 3. Prepare 1x sample diluent, 1X wash buffer and 1X conjugate as specified on page 3.

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

2. Pipet **100 uL standards A-G and diluted samples** in duplicate into appropriate wells. Mix gently, and incubate at **room temperature** (20-25oC) for **30 minutes**.

3. Remove or aspirate the plate contents and **wash the wells 3 times** with 300 uL of distilled or deionized water using an automated washer. If washing manually then dump the plate contents and tap over paper towels, add water, shake the contents of 5-10 seconds and repeat the steps. Tap the plate over fresh paper towels between each washing.

5. Pipet **100 uL of 1x enzyme conjugate** into each well. Mix gently, and incubate for **20 minutes** at room temperature as in step 3.

6 **Wash the wells 4 times** as in step 4. Tap the plate over fresh paper towels to remove traces of liquid from the last washing step.

7. **Add 100 uL of TMB Substrate** into each well. Mix gently. Cover the plate and incubate in the dark for **10 minutes** at room temperature. Blue color develops. 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 then reduce the incubation time.

8. Stop the reaction by adding **100 uL of stop solution** to all wells. Mix gently. 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.