ELISA kits available from ADI:

Catalog#	ProdDescription
600-400-CTN	Dog Cardiac Troponin 1 (Tn-I) ELISA Kit
600-410-CTN	Human Cardiac Troponin 1 (Tn-I) ELISA Kit
600-420-CTN	Monkey Cardiac Troponin 1 (Tn-I) ELISA Kit
600-430-MTN	Monkey Skeletal Muscle Troponin1 (Tn-I) ELISA Kit
600-440-CTN	Mouse Cardiac Tn-I ELISA kit for plasma samples
600-450-CTN	Mouse Cardiac Troponin 1 (Tn-I) ELISA Kit
600-470-CTN	Pig Cardiac Troponin 1 (Tn-I) 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
6240	Mouse Serum Amyloid A ELISA Kit
6490	Rat α-1-Acid Glycoprotein 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-6240-10

Monkey Serum Amyloid (SAA)

ELISA KIT Cat. # 6240-10

For Quantitative Determination of Serum Amyloid A (SAA) in Monkey Serum

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

Monkey α-1 Acid Glycoprotein (SAA) ELISA
KIT Cat. No. 6240-10

Kit Components, 96 tests	
Anti-Monkey Cardiac SAA coated strip plate (8 wells x 12 strips) #6240-10-1	1 plate
SAA Reference Standard, 0.20 ml, Lyophilized, #6240-10-2	1 vial
Anti-SAA-HRP Conjugate, 11 ml # 6240-10-3	1 bottle
Diluent, 50 ml #6240-10-4	1 bottle
Wash Solution (20X), 50 ml #6240-10-WB	1 bottle
TMB Substrate, 11 ml #6240-10-TMB	1 bottle
Stop solution, 11 ml #6240-10-SS	1 bottle
Instruction Manual # M-6240-10	1 manual

INTRODUCTION

.

Human Serum Amyloid A protein-1 (SAA1) is a multifunctional apolipoprotein produced by hepatocytes in response to proinflammatory cytokines. It is secreted as a 12 kDa, 104 aa, nonglycosylated polypeptide that displaces apoA1 in the HDL3 complex. The SAA1 gene is one of three SAA genes in human, and it shows multiple alleles that are race dependent. The SAA1 gene product differs from the SAA2 gene product by only seven amino acids. Circulating SAA1 shows multiple proteolytically-generated isoforms, with anywhere from one-to-three amino acids being cleaved from either the N- or C-terminus. Mature human SAA1 is 72%, 82% and 72% aa identical to mature mouse, rabbit and hamster SAA1, respectively.

SAA is an acute phase serum protein that is elevated in mice approximately 50-fold following lipopolysaccharide (LPS) injection. In mice, two major forms of SAA are induced during the acute phase response, SAA1 and SAA2. Studies have shown that the two forms are similarly increased in response to different inflammatory stimuli. This ELISA kit uses antibodies that preferentially detect SAA2. Measurement of SAA provides a useful biomarker of inflammation and disease.

ADI's Monkey SAA ELISA provides a rapid, specific and sensitive assay for measuring SAA in serum or other biological fluids.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate SAA concentrations. Read off the SAA concentrations of the control and patient samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:10K then the values must be multiplied by 10,000 and results are expressed as ug/ml.

If available, graphing software may be used to analyze the data. Depending on the range of the standard curve used, we find that good fits of the data may be obtained with linear regression analysis or using a two-site binding model. Alternatively, standard curves may be generated using a point-to- point fit.

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 SAA concentration detectable using this assay is below 0.8 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.

Specificity: The antibodies used in this kit are specific for Monkey SAA and have shown no cross-reactivity with other proteins.

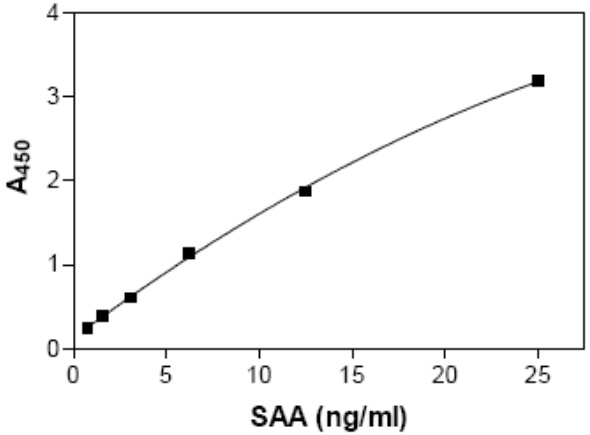
Species Crossreactivity: Cross-reactivity of Monkey SAA ELISA kit with other animals has not been tested.

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	**Neg. Control**	0 ng/ml	0.084	
B1, B2	**Standard A**	0.78 ng/ml	0.252	
C1, C2	**Standard B**	1.56 ng/ml	0.384	
D1, D2	**Standard C**	3.13 ng/ml	0.609	
E1, E2	**Standard D**	6.25 ng/ml	1.139	
F1, F2	**Standard E**	12.5 ng/ml	1.870	
G1, G2	**Standard F**	25 ng/ml	3.182	
H1, H2	**Sample 1**		0.625	3.2 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.



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

PRINCIPLE OF THE TEST

Monkey SAA ELISA kit is based on binding of Monkey SAA 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 SAA 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 SAA 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

The Monkey SAA ELISA Kit is for research use only.

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, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION and HANDLING

Collect blood by venipuncture; allow clotting, and separating the serum by centrifugation at room temperature. If sera cannot be immediately assayed, **store frozen at -70oC** for up to six months. Avoid repeated freezing and thawing of samples. **Cell or tissues extract samples have not been optimized.**

REAGENT PREPARATION

1. **Wash Buffer (20X)**. Dilute the entire 50 ml with 950 ml of distilled or deionized water (total volume 1000 ml). Store at room temperature..
2. **Reference Standard:** Reconstitute as shown on page 3.

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 SAA reference standard should be stored at -20oC.

PREPARATION & DILUTION OF SAMPLES

Denaturation:

1. For each sample pipet 100 uL of serum into a polypropylene microcentrifuge tube.
2. Incubate the samples in a 60oC water bath for 60 minutes.

Dilution:

3. After denaturation, dilute 2.0 uL of sample with 498 uL of diluent to give a 250-fold dilution.

TEST PROCEDURE

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

1. Reconstitute the lyophilized reference standard with 200 uL of distilled or deionized water. The concentration **of the reference standard is given on the vial. Immediately aliquot and store** any unused reference standard at –20oC or below. Do not heat inactivate the reference standard.

2. Prepare liquid standards using the following dilution scheme. Label 8 microcentrifuge tubes as 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0 ng/ml.

3. For standard F (25 ng/ml) pipet diluent and add reconstituted SAA reference standard as indicated on the vial. Mix gently. Prepare the remaining standards as shown below.

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 dilution scheme, you will have 250 uL of negative and all standards (B-F), and 500 uL of Std. A. You would need 200 uL of each standard (100 uL in duplicate.

Monkey derived products are controlled by international import/export restrictions. To comply with these restrictions ADI supplies the Monkey α-1-SAA reference standard with this ELISA kit of a non-monkey origin. The standard curve obtained with this material is identical to that obtained with monkey MMY standard.

Monkey SAA Stds	Stock Volume	SAA diluent	Final Volume
Std F (25 ng/ml)	500 uL	0	500 uL
Std E (12.5 ng/ml)	250 uL of Std F	250 uL	500 uL
Std D (6.25 ng/ml)	250 uL of Std E	250 uL	500 uL
Std C (3.13 ng/ml)	250 uL of Std D	250 uL	500 uL
Std B (1.56 ng/ml)	250 uL of Std C	250 uL	500 uL
Std A (0.78 ng/ml)	250 uL of Std B	250 uL	500 uL
Negative (0 ng/ml)	0	250 uL	250 uL

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

4. Pipet **100 ul standards and samples** into appropriate wells.

5. Pipette **100 ul of anti-SAA-HRP conjugate** into each well. Mix gently, and incubate at room temperature (20-25oC) for **60 minutes on an orbital shaker (100-150 rpm.** If an automated shaker is not available, the plate can be mixed manually every few minutes.

6. Remove or aspirate the plate contents and **wash the wells 5-6 times** with 300 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.

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. 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. 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.

10. 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.