Human SAA ELISA Kit

For the quantitative determination of human serum amyloid A (SAA) concentrations in serum, plasma, cell culture supernatant and other biological fluids.

Catalogue Number: EL10015

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR DIAGNOSTIC USE

ANOGEN

2355 Derry Road East, Unit 23
Mississauga, Ontario
CANADA L5S 1V6
Tel: (905) 677-9221 or (877) 755-8324
Fax: (905) 677-0023

Email: info@anogen.ca ♦ Web Site: www.anogen.ca

S7.5 (03) SAA

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INTENDED USE

This Human SAA ELISA Kit is to be used for the *in vitro* quantitative determination of human serum amyloid A (SAA) concentrations in serum, plasma, cell culture supernatant, and other biological fluids. This kit is intended FOR LABORTORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

Serum Amyloid A (SAA) is an acute-phase protein. During acute events, the rise in SAA levels is the most rapid and intense increase of all acute phase proteins. Cytokines such as IL-1, IL-6, and TNF are considered mediators of SAA protein synthesis. They stimulate hepatocytes in the liver to produce and release SAA into the bloodstream. When elevated above normal levels SAA is almost exclusively bound to High Density Lipoproteins (HDL), causing SAA to behave like an apolipoprotein - a protein moiety occurring in plasma lipoprotiens. SAA circulates at trace levels (1-5 μg/mL) during normal conditions; however 4-6 hours after inflammatory stimulus, SAA levels can increase by as much as 1000 fold to remarkably elevated levels (500-1000 μg/mL), thus making SAA a sensitive marker. [1,2]

Structural analysis revealed this 104 amino acid (a.a.) polypeptide in its native state has a molecular mass of 12-14 kDa. Serum amyloid A is the serum precursor of amyloid A (AA) protein (8.5 kDa), which is formed when the first 76 a.a.'s of SAA are cleaved. The human SAA protein is polymorphic being made up of a family of several related proteins (SAA_1 to SAA_4). SAA genes are located on chromosome 11p.[1] SAA_1 and SAA_2 are similar genes, which differ by 7 amino acids or more, and encode acute-phase SAA's. SAA_3 appears to be a pseudogene and is substantially different from the others. SAA_4 does not vary significantly during the acute phase response and is an isoform that is present on HDL during homeostasis.[3,4] Each of the acute phase proteins have a unique function in modulating host immune responses but the role of SAA remains unclear. It is known that HDL inhibits SAA's function. This suggests that SAA needs to be released from HDL complexes in order to become active.[5] Recently it was reported that SAA may have an important pro-inflammatory and immunostimulating role by recruiting neutrophils, monocytes, and T-lymphocytes into inflammatory lesions.[5,6] As a result of SAA's association with HDL, a role in cholesterol metabolism has been proposed. SAA, after dissociation from HDL, may play a role in cholesterol transport at local tissues sites during inflammation by binding cholesterol.[2,7]

High levels of SAA can be seen in patients with acute and chronic inflammation. Secondary amyloidosis may develop as a result prolonged or repeated inflammatory conditions in which SAA levels remain elevated. This progressive, fatal condition is characterized by a gradual loss of organ function, in which fibrils are deposited in peripheral tissues and major organs. The fibrils are caused by the incomplete degradation of SAA in which the AA fragment (8.5 kDa) from the original SAA protein has been enzymatically cleaved. Measuring SAA levels in these patients may be a useful indicator of degree of inflammation and response to therapy. Inflammatory disorders such as rheumatoid arthritis, juvenile arthritis, ankylosing spondylitis, familial mediterranean fever, progressive sclerosis as well as chronic infections such as tuberculosis and osteomyelitis are predisposed to developing amyloidosis.[8,9] Measuring

SAA levels is also significant in determining pulmonary inflammation in patients with cystic fibrosis,[10] diagnosing and predicting renal allograft rejection,[11] determining anti-microbial therapy response in urinary tract infections,[12] opportunistic infections in AIDS,[13] inflammation in acute viral infections,[14] biocompatiblility of hemodialysis,[15] tissue damage in post-acute myocardial infarction, [17] and the outcome in severe unstable angina.[16] Also, a differential diagnosis of inflammatory disease may be employed by measuring SAA levels. Acute viral infections may be distinguished from bacterial infections by determining SAA levels.[14-17] It may be useful to confirm diagnosis of acute viral diseases if SAA is assayed at the same time as C-reactive protein, which is a useful inflammatory marker for bacterial infections and does not rise during viral disease.[16]

This SAA ELISA is a 2.5-hour solid phase immunoassay readily applicable to measure SAA in serum, plasma, cell culture supernatant, and other biological fluids in the range of 0 to 80 ng/mL. It showed no cross reactivity with other cytokines tested. This SAA ELISA is expected to be effectively used for further investigations into the relationship between SAA and the various conditions mentioned.

PRINCIPLE OF THE ASSAY

This SAA enzyme-linked immunosorbent assay (ELISA) applies a technique called quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for SAA. Standards or samples are then added to the appropriate microtiter plate wells and incubated. SAA if present, will bind and become immobilized by the antibody pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove any unbound SAA and other components of sample. In order to quantitate the amount of SAA present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated monoclonal antibody specific for SAA is added to each well to "sandwich" the SAA immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugated antibodies and a TMB (3,3'5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate solution are allowed to react over a short incubation period. Only those wells that contain SAA and enzyme-substrate reaction will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the colour change measured spectrophotometrically at a wavelength of 450 nm ± 2 nm.

In order to measure the concentration of SAA in the samples, this kit includes two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant testing). According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D) versus SAA (ng/mL). The concentration of SAA in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

	96 tests

1. **SAA MICROTITER PLATE** (Part EL15-1) ***96 wells***
   Pre-coated with anti-human SAA monoclonal antibody.

2. **SAA CONJUGATE** (Part EL15-2) ***15 mL***
   Anti-human SAA monoclonal antibody conjugated to horseradish peroxidase with preservative.

3. **SAA STANDARD** (Part EL15-3) ***2 vials***
   Human SAA (160 ng/vial) in a buffered protein base with preservative, lyophilized.

4. **CALIBRATOR DILUENT I** (Part EL15-4) ***30 mL***
   Animal serum with preservative. *For serum/plasma testing*.

5. **CALIBRATOR DILUENT II** (Part EL15-5) ***30 mL***
   Cell culture medium with calf serum and preservative. *For cell culture supernatant testing.*

6. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

7. **SUBSTRATE A** (Part EL15-6) ***11 mL***
   Buffered solution with H_2O_2.

8. **SUBSTRATE B** (Part 30007) ***11 mL***
   Buffered solution with TMB.

9. **STOP SOLUTION** (Part 30008) ***14 mL***
   2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100μL and 50-200μL for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm).
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Wastes: Autoclave for 60 minutes at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the virus before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form, which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

1. **COLLECTION, HANDLING AND STORAGE**
   a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
   b) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.
   c) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection there must be quick separation of plasma with less than 30 minutes on ice. Centrifuge for 10 minutes (4°C) to remove any particulate. *This SAA ELISA kit is not affected by haemolysis of specimens. No adverse effects have been noted in the presence of anti-coagulants, sodium citrate, EDTA, or heparin*.

   - Avoid grossly hemolytic, lipidic or turbid samples.
   - Serum, plasma, and cell culture supernatant samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
   - When performing the assay slowly bring samples to room temperature.
   - It is recommended that all samples be assayed in duplicate.
   - DO NOT USE HEAT-TREATED SPECIMENS.

2. **DILUTION PROCEDURES**
   a) **Serum/plasma samples:**
   Samples require a final dilution of 1: 50 in order to detect normal SAA levels. This dilution can be adjusted according to the range of the standard curve**.** Dilute samples with Calibrator Diluent I. The suggested dilution procedure is as follows:

   Step1: 20µL serum/plasma sample + 180 µL Calibrator Diluent I. Mix well. (1:10)
   Step2: 50µL dilution (from step1) + 200 µL Calibrator Diluent I. Mix well. (1:50)

   b) **Cell culture supernatant samples:**
   For the cell culture supernatant sample, the minimum detectable level is 0.6 ng/mL.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of

distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

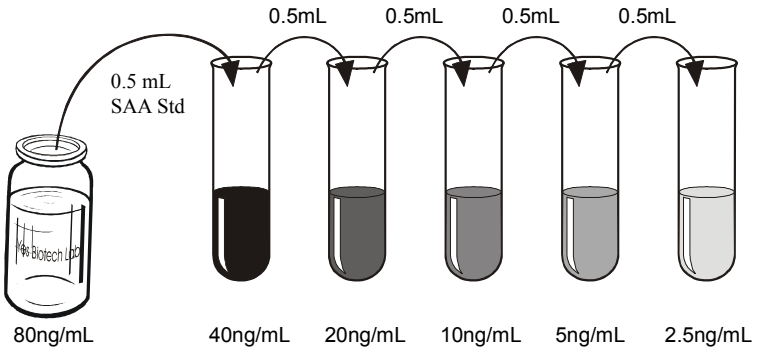
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	1.5	1.5	3.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **SAA Standard:**

a) Two vials of Standard are provided in this kit to allow both serum/plasma and cell culture supernatant testing. Reconstitute SAA Standard with either 2.0 mL of Calibrator Diluent I (for serum/plasma testing) or Calibrator Diluent II (for cell culture supernatant testing). This reconstitution produces a stock solution of 80 ng/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The SAA standard stock solution can be stored frozen (-20°C) for up to 30 days. Avoid freeze-thaw cycles; aliquot if repeated use is expected.

b) Use the above stock solution to produce a serial 2-fold dilution series within the range of this assay (2.5 ng/mL to 80 ng/mL) as illustrated below. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer be sure to mix contents thoroughly. The undiluted SAA Standard will serve as the high standard (80 ng/mL) and the Calibrator Diluent will serve as the zero standard (0 ng/mL).



ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and SAA Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards and Samples to the Microtiter Plate.*

Wells	Contents	**Wells**	Contents
1A, 1B	Standard 1 - **0 ng/mL** (S1)	**2A, 2B**	Standard 5 - **20 ng/mL** (S5)
1C, 1D	Standard 2 - **2.5 ng/mL** (S2)	**2C, 2D**	Standard 6 - **40 ng/mL** (S6)
1E, 1F	Standard 3 - **5 ng/mL** (S3)	**2E, 2F**	Standard 7 - **80 ng/mL** (S7)
1G, 1H	Standard 4 - **10 ng/mL** (S4)	**2G-12H**	SAA samples

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	S1	S5	2	6	10	14	18	22	26	30	34	38
B	S1	S5	2	6	10	14	18	22	26	30	34	38
C	S2	S6	3	7	11	15	19	23	27	31	35	39
D	S2	S6	3	7	11	15	19	23	27	31	35	39
E	S3	S7	4	8	12	16	20	24	28	32	36	40
F	S3	S7	4	8	12	16	20	24	28	32	36	40
G	S4	1	5	9	13	17	21	25	29	33	37	41
H	S4	1	5	9	13	17	21	25	29	33	37	41

2. Add 100 μL of Standard or Sample to the appropriate well of the antibody pre-coated wells of the Microtiter Plate. Cover and incubate for 1 hour at room temperature.

3. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μL/well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

4. Add 100μL of conjugate to each well. Cover and incubate for 1 hour at room temperature.

5. Prepare Substrate Solution (see Preparation of Reagents) no more than 15 minutes before end of second incubation.

6. Repeat wash procedure as described in Step 3.

7. Add 100 μL Substrate Solution to each well. Cover and incubate for 15 minutes at room temperature.

8. Add 100 μL Stop Solution to each well. Mix well.

9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of SAA in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding SAA concentration (ng/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 ng/mL) or (S1) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of SAA in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding SAA concentration. If samples generate values higher than the highest standard, dilute the samples and repeat the assay, the concentration read from the standard curve must be multiplied by the dilution factor.

3. To determine the final concentration of SAA in serum or plasma samples, the concentration read from the standard curve must be multiplied by the *dilution factor (50).*

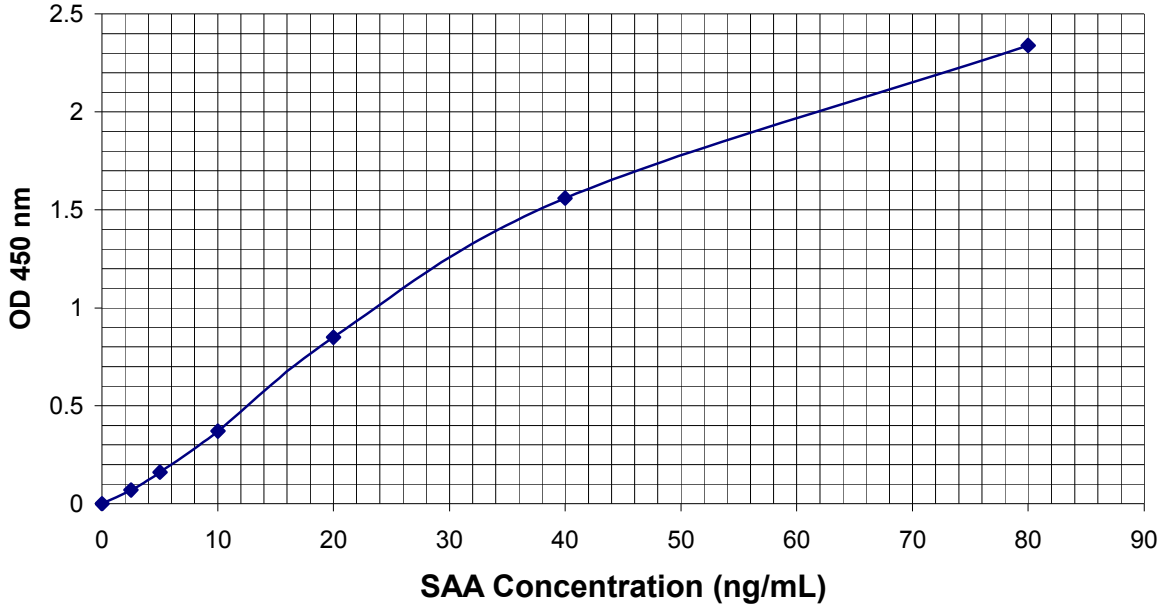
TYPICAL DATA

Results of a typical standard run of a SAA ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of *illustration only*, and should not be used to calculate unknowns. Each user should obtain its own standard curve.

EXAMPLE ONE

The following data was obtained for a standard curve using Calibration Diluent I.

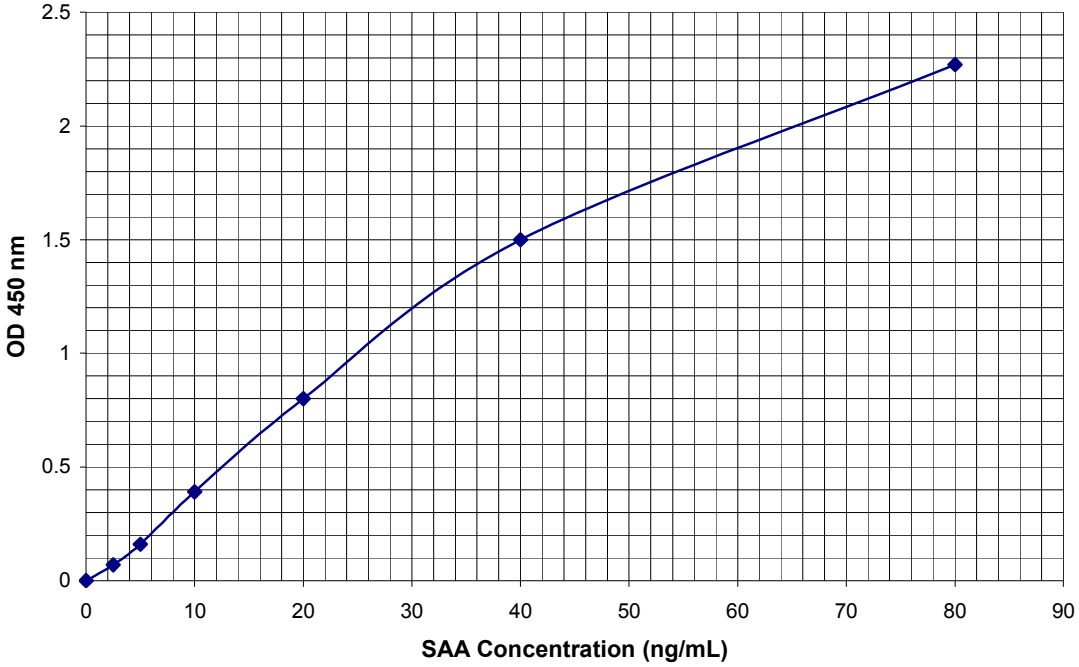
Standard (ng/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) - (S1)
0	0.039, 0.040	0.039	0
2.5	0.108, 0.106	0.107	0.07
5	0.197, 0.197	0.197	0.16
10	0.410, 0.417	0.413	0.37
20	0.894, 0.880	0.877	0.85
40	1.567, 1.627	1.597	1.56
80	2.439, 2.320	2.379	2.34



EXAMPLE TWO

.The following data was obtained for a standard curve using Calibration Diluent II.

Standard (ng/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) - (S1)
0	0.037, 0.037	0.037	0
2.5	0.114, 0.096	0.105	0.07
5	0.186, 0.211	0.198	0.16
10	0.427, 0.422	0.424	0.39
20	0.823, 0.858	0.840	0.80
40	1.531, 1.528	1.529	1.50
80	2.237, 2.384	2.310	2.27



PERFORMANCE CHARACTERISTICS

1. **INTRA-ASSAY PRECISION**
   To detemine within-run precision, three different samples of known concentration were assayed by using 20 replicates in 1 assay.

	Calibrator Diluent I Assay			*Calibrator Diluent II Assay*		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	7.16	28.8	85.3	6.90	28.4	69.3
Standard Deviation (ng/mL)	0.20	1.18	2.40	0.36	0.85	4.17
Coefficient of Variation (%)	*2.8*	*4.1*	*2.8*	*5.3*	*3.0*	*6.0*

2. **INTER-ASSAY PRECISION**
   To determine between-run precision, three different samples of known concentration were assayed by replicates on 16-20 different assays.

	Calibrator Diluent I Assay			*Calibrator Diluent II Assay*		
Sample	1	2	3	1	2	3
n	20	20	20	16	16	16
Mean (ng/mL)	5.11	20.80	64.00	4.74	19.60	61.10
Standard Deviation (ng/mL)	0.53	1.66	3.82	0.37	1.44	4.85
Coefficient of Variation (%)	*10.3*	*8.3*	*6.0*	*7.7*	*7.3*	*7.9*

3. **RECOVERY**
The recovery of SAA spiked to 3 different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Average Recovery %	Range %
Cell culture media	98.2	95.0 – 105.0
Serum	104.0	102.2 – 107.0
Plasma	104.8	103.3 – 106.9

4. **SENSITIVITY**
The minimum detectable dose of SAA was determined by adding two standard deviations to the mean optical density value of 20 zero standard replicates and calculating the corresponding concentration from the standard curve. The minimum detectable dose of human SAA using a standard curve generated with Calibrator Diluent I is *1.1 ng/mL* and using Calibrator Diluent II is *0.6 ng/mL*.

5. **SPECIFICITY**
This ELISA recognises both natural and recombinant human SAA. This kit exhibits no detectable cross-reactivity with human; IL-8, IL-1ß, MCAF, FGF, TGF-ß, EGF, GM-CSF, M-CSF, MCP-3, TNF-α, RANTES, and EPO.

6. **CALIBRATION**
This immunoassay is calibrated against NIBSC SAA 1st international standard (code 92/680).

7. **EXPECTED NORMAL VALUES**
Fourteen apparently healthy, normal individuals were evaluated in this assay. The SAA concentration of serum/plasma samples is ranged from 1000-5000 ng/mL and urine samples is less than 2.5 ng/mL.

REFERENCES

1. Skinner, M. (1992) *J. Internal Med*. 232: 514-523.
2. Kisilevsky, R. (1991) *Med. Hypoth*. 35:3 37-41.
3. Malle, E.et al.(1993) *Atheroscl.* 102: 131-46.
4. Bausserman, L.L. et al.(1988) *Eur. J. Clin. Invest*. 18 :619-26.
5. Baldolato, R.et al. (1994) *J. Exp. Med*. 180:0.
6. Xu, L.et al. (1995) *J. Immunol*. 155: 0000-0000.
7. Liang, J.et al. (1995) *J. Lipid Res*. 36: 37-46.
8. Knecht, A.et al. (1985) *Ann. Int. Med.* 102(1): 71-72.
9. Reinhoff J.R, H.Y.et al. (1990) *Mol. Bio. Med.* 7: 287-98.
10. Marhaug, G.et al. (1983) *Acta. Peadiatr. Scand*. 72: 861-6.
11. Casl, M.T.et al. (1995) *Nephron*. 70: 112-113.
12. Casl, M.T.et al. (1993) *Ann. Clin. Biochem*. 30: 272-7.
13. Husebekk, A.et al. (1986*) Scand J. Infec. Dis*. 18: 389-394.
14. Nakayama, T.et al. (1993) *Clin. Chem*. 39(2): 293-7
15. Honkanen, E.et al. (1991) *Nephron*. 57: 283-7.
16. Hiroyuki, M. et al. (1993) *Arch Dis Child*. 68: 210-4.
17. Thomson, D. et al. (1992) *Ann. Clin. Biochem*. 29: 123-131.
18. Luizzo, G. et al. (1994) *N. Eng. J. Med*. 331: 417-24
19. Casl, M.T.et al. (1995) *Ann. Clin. Biochem*. 32: 196-200.

CITATIONS

1. Muriel Lavie et al. (2006) *Hepatology*. Volume 44, Issue 6, page 1626-1634.
2. Steven Bozinovski et al. *Am. J. Respir. Crit. Care Med.* Vol. 177, No. 3 (2008), pp. 269-278.
3. Sander I. van Leuven et al. (2007) *Journal of Lipid Research* Volume 48. 2640-6.
4. Smith DJ et al. (2011) *Eur. Respir. J.* 37(3):516-22.
5. Sukkar, MB (2012) *Eur. Respir. J.* 39(3):721-9.
6. Patricia G. Wilson et al. *Am J Pathol.* Dec 2008; 173(6): 1902–1910.
7. Qiulin Liao et al. *Clin Exp Metastasis.* Jun 2008; 25(4): 465–476.
8. Hutchinson AF et al. *Influenza Other Respir Viruses.* 2010 Jan; 4(1):33-9.
9. Nelson TL et al. *Cytokine.* 2007 May; 38(2):101-6.
10. Johannes HM Levels et al. *Proteome Sci.* 2011; 9: 34.
11. Wu TL et al. *Clin Chim Acta.* 2007 Feb; 376(1-2):72-6.
12. Leo Stockfelt et al. *Inhal Toxicol.* Jul 2013; 25(8): 417–425.
13. José L López-Campos et al. *BMC Med Res Methodol.* 2012; 12: 40.
14. Anna A Richards et al. *Am J Hum Biol.* Jul 2013; 25(4): 457–464.
15. Guojun Zhang et al. *Oncol Lett.* Apr 1, 2012; 3(4): 940–944.