

Human SAA ELISA Kit (Second Generation)

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

Catalogue Number: EL10015L

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR DIAGNOSTIC USE



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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, and other biological fluids. This kit is intended FOR LABORATORY 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 lipoproteins. SAA circulates at trace levels (1-7 $\mu\text{g/mL}$) 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 $\mu\text{g/mL}$), 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 amino acids of amyloid A protein are cleaved. The human SAA protein is polymorphic being made up of a family of several related proteins (SAA₁ to SAA₄). SAA genes are located on chromosome 11p.¹ SAA₁ and SAA₂ are similar genes, which differ by 7 amino acids or more, and encode acute-phase SAAs. SAA₃ appears to be a pseudogene and is substantially different from the others. SAA₄ 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 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 biocompatibility 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, and other biological fluids in the range of 0 to 16µg/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, 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 EL15L-1) **96 wells**
Pre-coated with anti-human SAA monoclonal antibody
2. **SAA CONJUGATE** (Part EL15L-2) **15 mL**
Anti-human SAA monoclonal antibody conjugated to horseradish peroxidase with preservative
3. **SAA STANDARD** (Part EL15L-3) **2 vials**
Human SAA (160ng/vial) in a buffered protein base with preservative, lyophilized.
4. **CALIBRATOR DILUENT I** (Part EL15L-4) **60 mL**
Animal serum with preservative, for standard and sample dilution
5. **SAA QUALITY CONTROL SAMPLE I** (Part EL15L-5) **1 vial**
Lyophilized, pre-diluted quality control sample for serum/plasma testing. The control sample is calibrated against SAA WHO 1st international standard (92/680).
6. **SAA QUALITY CONTROL SAMPLE II** (Part EL15L-6) **1 vial**
Lyophilized, pre-diluted quality control sample for serum/plasma testing. The control sample is calibrated against SAA WHO 1st international standard (92/680).
7. **WASH BUFFER (20X)** (Part 30005) **60 mL**
20-fold concentrated solution of buffered surfactant.
8. **SUBSTRATE A** (Part EL15L-7) **11 mL**
Buffered solution with H₂O₂.
9. **SUBSTRATE B** (Part 30007) **11 mL**
Buffered solution with TMB.
10. **STOP SOLUTION** (Part 30008) **14 mL**
2N Sulphuric Acid (H₂SO₄). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100 μ L and 50-200 μ L 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 \pm 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. 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) **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.
- b) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, heparin or CDPA 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.
 - Avoid hemolytic, lipidic or turbid samples.
 - Serum and plasma samples to be used within 24-48 hours may be stored at 2-8°C. Otherwise, samples must be 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. SAMPLE DILUTION

- Normal serum/plasma samples should be diluted 100 times with SAA Calibrate Diluent I to determine SAA levels. Agitate samples briefly with vortex prior to the dilution to ensure homogeneousness of the sample. If samples are frozen, thaw sample and spin down the moisture inside the sealed sample tubes before agitating samples. Pipette 10µl of sample into 990µl of SAA Calibrate Diluent I precisely, and mix well.
- For tested samples with value higher than or close to the highest standard, make a 10 fold further dilution from the 1:100 diluted sample by mixing 50µl of 1:100 diluted sample with 450µl SAA Calibrate Diluent I and repeat the assay. Dilute the 1:1,000 diluted sample 10 fold further in the same way with SAA Calibrate Diluent I and repeat the assay if tested value is still higher than or close to highest standard. In very rare situation, an additional 10 fold dilution is needed to make the diluted sample within assay range.

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.

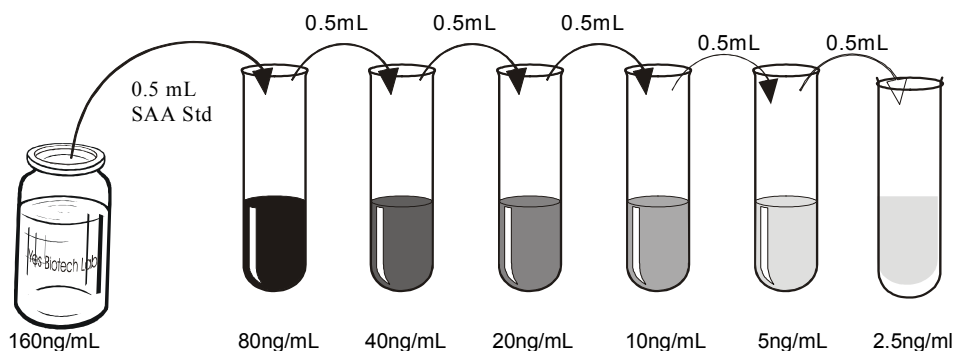
1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable 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) Reconstitute SAA Standard with 1.0mL of Calibrator Diluent I for serum/plasma testing. This reconstitution produces a stock solution of 160ng/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 2-fold dilution series within the range of this assay (2.5ng/mL to 160ng/mL) as illustrated below. Add 0.5mL Calibrator Diluent I 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 (160ng/mL) and the Calibrator Diluent will serve as the zero standard (0ng/mL).



4. **SAA Quality Control Samples:** Add 0.5mL of deionised water into each vial. Inspect to make sure that the pellet inside the glass vial is completely dissolved. Allow the vial to set for 10mins and gently agitate the solution inside before adding sample to ELISA plate. The reconstituted quality control samples should be used immediately. No further dilution is needed for QC samples. The established average value and standard deviation of the samples are indicated on the label of the vials as reference.

ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and SAA Standards before starting assay procedure (see Preparation of Reagents). *The table and diagram provided below can 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 – 2.5ng/mL (S2)			2C, 2D	Standard 6 - 40 ng/mL (S6)		
1E, 1F	Standard 3 – 5ng/mL (S3)			2E, 2F	Standard 7 - 80 ng/mL (S7)		
1G, 1H	Standard 4 – 10ng/mL (S4)			2G, 2H	Standard 8 – 160 ng/mL (S8)		
3A, 3B	Control level 1 (QC1)			3C, 3D	Control level 2 (QC2)		
3E, 12H	Samples						

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S5	QC1	3	7	11	15	19	23	27	31	35
B	S1	S5	QC1	3	7	11	15	19	23	27	31	35
C	S2	S6	QC2	4	8	12	16	20	24	28	32	36
D	S2	S6	QC2	4	8	12	16	20	24	28	32	36
E	S3	S7	1	5	9	13	17	21	25	29	33	37
F	S3	S7	1	5	9	13	17	21	25	29	33	37
G	S4	S8	2	6	10	14	18	22	26	30	34	38
H	S4	S8	2	6	10	14	18	22	26	30	34	38

2. Add 100µL 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: 350-400µL). 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 μ L Substrate Solution to each well. Cover and incubate for **15 minutes at room temperature**.
8. Add 100 μ L Stop Solution to each well. Mix well.
9. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 10 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. Construct the standard curve using graph paper or statistical software.
2. To manually 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 in sample.
3. If statistic software (program) is available, it is recommended to select "Point to Point" or "Four Parameter Logic" method to obtain the standard curve for accurate sample value determination.
4. To determine the concentration of SAA in serum or plasma samples, the concentration read from the standard curve must be multiplied by the dilution factor (100).
5. If a tested sample generates value higher than or close to the highest standard, make a 10 fold further dilution from the diluted samples with SAA Calibrator Diluent I (see page 6), and repeat the assay. If the further diluted sample still generates value higher than or close to the highest standard, repeat the above procedure until a result is obtained within assay range. Then, the measured value is multiplied with dilution factors to obtain the final concentration for the sample.
6. It is suggested to run the SAA quality control samples with each assay. If the measured values of the QC samples are deviated more than ± 2 SDs from the established average value, it is recommended that the assay should be repeated. It is also recommended that each lab may establish its own quality control stringency according to assay results.

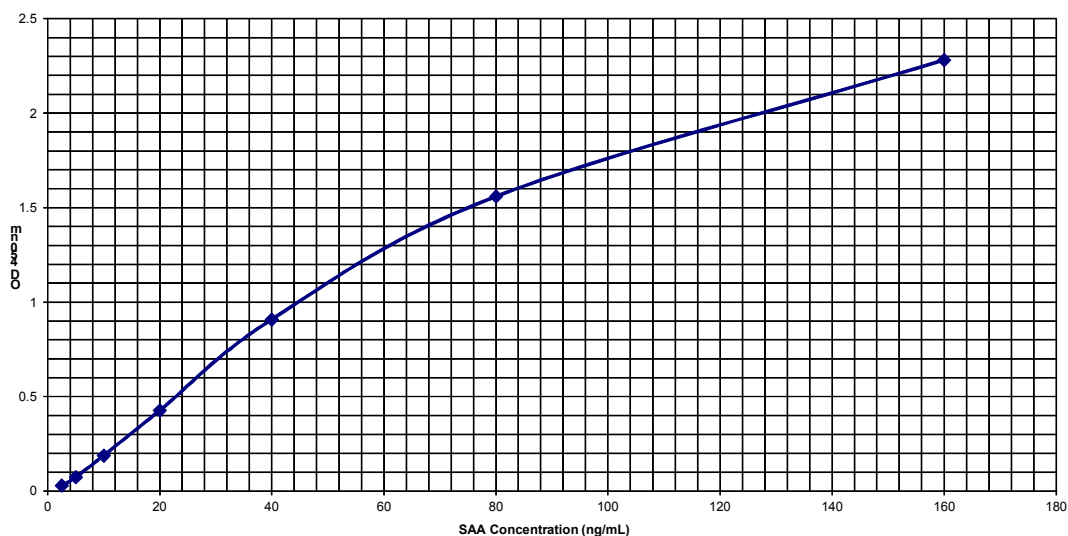
TYPICAL DATA

1. STANDARD CURVE

Typical data of a test run with SAA standard curve are shown below. Variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain its own standard curve.

The example is for the purpose of *illustration only*, and should not be used to calculate unknowns.

Standard (ng/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted (Std.) - (S1)
0	0.041, 0.041	0.0410	0
2.5	0.071, 0.068	0.0695	0.0285
5	0.115, 0.113	0.1140	0.0730
10	0.237, 0.221	0.2290	0.1880
20	0.463, 0.471	0.4670	0.4260
40	0.956, 0.942	0.9490	0.9080
80	1.583, 1.616	1.5995	1.5585
160	2.291, 2.354	2.3225	2.2815



3. QUALITY CONTROL SAMPLES

It is recommended to run quality control samples with standard curve. The expected quality control sample ranges (mean \pm 2SD) are determined by established average value and standard variation of the quality control samples. Refer to the information on the labels of each lot of the quality control samples to calculate. The following

data are from an assay of the QC samples obtained with **Point to Point** curve fitting method.

Standard (ng/ml)	OD450	Mean OD	Expected sample ranges (ng/ml)	Obtained values (ng/ml)
0	0.039 0.046	0.0425	NA	
2.5	0.079 0.083	0.0810	NA	
5	0.134 0.136	0.1350	NA	
10	0.257 0.262	0.2595	NA	
20	0.533 0.530	0.5315	NA	
40	1.065 1.106	1.0855	NA	
80	1.780 1.777	1.7785	NA	
160	2.429 2.336	2.3825	NA	
QC 1 (Mean=13.427, 2SD=2.996)	0.344 0.360	0.3520	10.431-16.424	13.401
QC 2 (Mean=85, 2SD=21.234)	1.764 1.783	1.7735	63.766-106.235	79.711

PERFORMANCE CHARACTERISTICS

1. INTRA-ASSAY PRECISION

To determine within-run precision, two diluted samples of known concentration were assayed by measuring 8 replicates in 1 assay.

Sample	1	2
N	8	8
Mean (ng/mL)	15.567	83.479
Standard Deviation (OD)	0.009	0.041
<i>Coefficient of Variation (%)</i>	2.532	3.512

2. INTER-ASSAY PRECISION

To determine between-run precision, two diluted samples of known concentration were measured in 8 different assays.

Sample	1	2
N	8	8
Mean (ng/mL)	12.65	84.60
Standard Deviation (ng/mL)	0.77	3.60
<i>Coefficient of Variation (%)</i>	6.10	4.18

3. INTRA-ASSAY ACCURACY

Intra-assay accuracy was validated by 10 repeated measurements of serial diluted samples at 5 concentration levels covering the calibration range in one assay. The intra-assay accuracy of the test was estimated by comparing the average value of the measured samples with nominal value.

Sample Dilution	Nominal value (ng/ml)	Point to Point curve fitting			Four Parameter Logic curve fitting		
		Measured values (ng/ml)	CV (%)	Percentage of nominal value (%)	Measured values (ng/ml)	CV (%)	Percentage of nominal value (%)
1:100	142.98	152.492	4.7	107	148.425	4.7	97.3
1:200	71.49	67.661	2.5	94.6	65.494	2.5	91.6
1:400	35.75	33.517	3.2	93.7	33.179	3.2	92.8
1:800	17.87	16.761	3.1	93.8	16.946	3.1	94.8
1:1600	8.935	8.266	4.2	92.5	8.306	4.2	92.9

4. INTER-ASSAY ACCURACY

Inter-assay accuracy was validated by analyzing serial diluted samples at 5 concentration levels in 8 runs of three days. The inter-assay accuracy of the test was estimated by comparing the average value of the measured samples with nominal value.

Sample Dilution	Nominal value (ng/ml)	Point to Point curve fitting			Four Parameter Logic curve fitting		
		Range of measured values (ng/ml)	CV (%)	Percentage (average) of nominal value (%)	Range of measured values (ng/ml)	CV (%)	Percentage (average) of nominal value (%)
1:100	142.98	119.90-152.49	8.6	96.7	109.27-148.42	7.6	90.9
1:200	71.49	63.845-75.460	6.5	100.5	60.785-74.07	5.7	97.6
1:400	35.75	32.750-37.890	5.4	98.8	32.215-37.757	4.9	97.9
1:800	17.87	16.761-20.156	4.6	103	17.35-20.086	4.9	103
1:1600	8.935	5.836-10.517	17	79.3	6.117-10.48	18	81.1

5. 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 %
Serum	104.0	102.2 – 107.0
Plasma	104.8	103.3 – 106.9

6. SENSITIVITY

The minimum detectable dose of SAA was determined by adding two standard deviations to the mean optical density value of 18 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 1.1ng/mL.

7. SPECIFICITY

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

8. CALIBRATION

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

9. EXPECTED NORMAL VALUES

Fourteen apparently healthy, normal individuals were evaluated in this assay. The SAA concentration of serum/plasma samples is ranged from 200-6500ng/mL and urine samples is less than 2.5ng/mL.

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