

The Serum Amyloid A (SAA) Turbidimetric Immunoassay Kit

Catalogue number: 51910

For the quantitative determination of Serum Amyloid A in human serum and plasma

This package insert must be read in its entirety before using this product Use only the current version of product data sheet enclosed with the kit

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FOR RESEARCH USE ONLY NOT FOR USE IN DIAGNOSTIC PROCEDURES

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Cat. No.	Size	Approximately tests
51910-05	R1: 15ml, R2: 5ml	100
51910-10	R1: 30ml, R2: 10ml	200
51910-20	R1: 60ml, R2: 20ml	400
51910-50	R1: 150ml, R2: 50ml	1000
51910-100	R1: 300ml, R2: 100ml	2000

INTRODUCTION

Serum Amyloid A (SAA) is a family of apolipoproteins associated with highdensity lipoprotein in plasma. There are 4 different isoforms of SAA, namely SAA1 to SAA4, encoded by 4 distinct genes. SAA are produced predominantly by the liver. The main functions of SAA include the transport of cholesterol to the liver for secretion into the bile, the recruitment of immune cells to inflammatory sites, and the induction of enzymes that degrade extracellular matrix.

SAA is an acute phase protein that is markedly increased in response to inflammatory stimulus. Similar to CRP, levels of acute-phase SAA increase within hours after inflammatory stimulus, and the magnitude of increase may be greater than that of C-reactive protein (CRP). Even relatively trivial inflammatory stimuli can lead to SAA responses. It has been suggested that SAA levels correlate better with disease activity in early inflammatory joint disease than do ESR and CRP. It returns rapidly to normal after resolution of inflammation. The normal range of circulating SAA is <10 mg/L.

PRINCIPLE OF THE ASSAY

This assay is a turbidimetric immunoassay for the quantitative measurement of SAA in human serum and plasma. A standard or sample is added into a cuvette and mixed with the reaction buffer R1. After a short incubation, the test reagent R2, which is a suspension of microparticles coated with SAA antibodies, is added into the cuvette and mixed. The presence of SAA in the standard or sample causes the immune-particles to aggregate. The extent to which the microparticles aggregate is quantified by the amount of light scattering measured as absorbance by a chemistry analyzer. The concentration of SAA in unknown samples can be interpolated from a reference curve using the standards provided.

REAGENTS SUPPLIED

R1-Reaction buffer, a ready-to-use buffer solution containing salt, polyether compound and preservative

R2 – Test reagent, a ready-to-use suspension of polymer microparticles coated with rabbit anti-SAA polyclonal antibodies in storage buffer

OTHER MATERIALS REQUIRED

- 1. Clinical chemistry analyzer
- 2. SAA calibrator, provided separately (Cat No: 51910-S1)
- 3. SAA quality controls, optional, provided separately (51019-C1)
- 4. Deionized water
- 5. Analyzer-specific reagent containers for R1 and R2

STORAGE

The kit should be stored at $2-8^{\circ}$ C upon receipt. Once opened, the reagents may be stored at $2-8^{\circ}$ C for up to 4 weeks.

SAMLE HANDLING

This kit can be used to determine SAA in human serum and plasma samples. Blood specimens should be collected aseptically into appropriate tubes. Plasma should be prepared by standard techniques for laboratory testing. The prepared specimens should be stored in closed vessels. If the assay cannot be performed within 24 hours or specimens are to be shipped, the specimens should be frozen at -20° C or below. For long-term storage of specimens, -70° C or below is recommended. To avoid freeze-thaw cycles, specimens should be aliquoted. Do not use hemolyzed, hyperlipemic, heat-treated or contaminated specimens. No dilution of the sample is required in this assay.

ASSAY PROCEDURE

Assay procedures may vary depending on the automated chemistry analyzer to be used. A general example of assay procedures is stated as follow:

- 1. Dispense 150µl of R1 into a clean cuvette
- 2. Add 1.5μl of serum or plasma samples, SAA calibrators or controls and incubate at 37°C for 5 minutes
- 3. Further add 50µl of R2
- 4. Read change of absorbance at Main Wavelength 570 nm for 8 minutes after the addition of R2
- 5. Calculate the concentration of SAA in unknown sample by interpolation from a reference curve using the standards provided

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each assay.

SAA (mg/L)	Absorbance(570 nm)	
0	-0.0040	
1	0.0055	
5	0.0270	
10	0.0607	
100	0.2366	
400	0.5540	



CALCULATION

- 1. Subtract the absorbance of the blank from that of standards and samples.
- 2. Generate a standard curve by plotting the absorbance obtained (y-axis) against SAA concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. 4-parameter curve fitting can be used for calculation.
- 3. Determine SAA concentration of samples from standard curve.

ASSAY CHARACTERISTICS

A. Sensitivity

The sensitivity is defined as the lower limit of detection and is estimated as the mean of the blank sample plus three times the SD obtained from the blank sample. The sensitivity of SAA assay is 0.123mg/L.

B. Precision

The precision of the SAA assay is < 10% CV. Two serum samples were assayed 20 times separately.

Sample	Mean SAA	SD	CV
-	(mg/L)	(mg/L)	
Sample 1	0.53	0.04	8.00%
Sample 2	15.79	0.77	5.00%

C. Linearity

The SAA assay is linear between 1 mg/L to 400 mg/L.

D. Interference

No interference was detected with hemoglobin up to 5 g/L, conjugated bilirubin up to 300 mg/L, free bilirubin up to 300 mg/L, and up to 5g/L lipid emulsion.