Human serum amyloid A (SAA)

Serum amyloid A apolipoprotein family consists of three members that in human beings are coded by different genes: SAA1, SAA2, and SAA4 (reviewed in 1-3). SAA1 and SAA2 are so-called acute phase isoforms. Their expression is increased in response to inflammation. SAA4 is a constitutive isoform, the expression of which does not change during an acute-phase response. In addition, one more related gene (SAA3) has been identified, although this gene is not expressed in human beings.

Biochemical properties of SAA

SAA1 and SAA2 are synthesized in the liver and secreted to the blood. When in the blood, SAA proteins form complexes with high density lipoproteins (HDL).

SAA1 and SAA2 genes have different alleles that give rise to three different SAA1 and two different SAA2 variants that differ in 1-3 amino acid residues. Both SAA1 and SAA2 consist of 104 amino acid residues. Along with full-length molecules, truncated SAA proteins lacking the N-terminal arginine have been found in human blood samples. The acute phase SAA1 and SAA2 proteins are highly homologous and have at least 97 identical amino acid residues. SAA4 consists of 112 amino acid residues. The homology between SAA4 and acute phase SAA isoforms is approximately 50%.

Recently, the crystal structure of human recombinant SAA1 containing an N-terminal histidine tag was published. It was reported that SAA contains four α-helix regions that span the amino acid residues 1–27, 32–47, 50–69, and 73–88 (4). In a solution, human recombinant SAA as well as purified endogenous SAA has a tendency to aggregate and form oligomers (4-6). Presumably, the association of SAA molecules is mediated by amino acid residues located within α-helix regions 1 (residues 2-8) and 3 (residues 52-59) (4).

The biological function of SAA

The biological function of SAA in inflammation is unclear. It has been suggested that SAA is involved in the recycling of cholesterol from damaged tissues. It might play the role of a signaling molecule that redirects HDL particles to activated macrophages and mediates the removal of stored cholesterol from them. Released cholesterol is then transferred to HDL to be used again in the membranes of new cells that are required during acute inflammation and tissue repair (7). Besides that, published studies demonstrate that recombinant SAA exhibits significant proinflammatory activity by inducing the synthesis of several cytokines and promoting chemotaxis for monocytes and neutrophils *in vitro* (1, 8). However, it is unclear as to whether SAA demonstrates similar properties under physiological conditions (9). Further research is required to elucidate the precise functions of SAA in physiological and pathological settings.

SAA as a diagnostic marker

SAA is a non-specific marker of inflammation, the concentration of which in blood increases in response to inflammatory stimuli such as tissue injury, infection or trauma. Similarly to C-reactive protein, SAA is a major acute phase protein in human beings. In normal conditions, SAA concentration in serum is approximately 1-10 μg/ml. However, during an acute-phase reaction, the concentration can rise to 1 mg/mL or even higher. The acute phase response usually lasts for several days and then the concentration of SAA gradually decreases in the absence of a new stimulus.

SAA can be used in diagnosis, predicting outcomes and assessing the efficacy of treatment in patients with inflammation. Specifically, it has been demonstrated in a number of studies that SAA concentration reflects the disease activity and grade of inflammation in patients with rheumatoid arthritis (10, 11). SAA is a sensitive biomarker of acute renal allograft rejection and it can be used to monitor SAA in kidney transplant patients for the early detection of acute rejection episodes (12, 13). In patients with myocardial infarction, SAA concentration is elevated to extremely high values and correlates with postinfarction complications and the mortality rate (14, 15). Elevated SAA concentrations were observed in patients with bacterial infections caused by different pathogens (16). In patients with urinary tract infections, the monitoring of the SAA level is useful for the evaluation of antimicrobial therapy efficiency (17).

Reagents for the development of SAA immunoassays

HyTest offers a set of mouse monoclonal anti-SAA antibodies that are suitable for the development of sandwich immunoassays for the quantitative detection of circulating SAA in human blood as well as for the immunodetection of SAA in Western blotting.

Monoclonal antibodies specific to human SAA

We provide eight mouse monoclonal antibodies (MAbs) for the detection of human SAA. They have been selected from among over 50 MAbs developed against human or canine SAA. All eight antibodies recognize recombinant human SAA1 isoform and endogenous SAA from human blood. The epitope of the MAb VSA25 is located in the region 23-29 aar; the epitope of the MAb VSA6 is located in the region 72-86 aar. The epitope specificities of other MAbs have not been established.

In addition to human SAA, provided antibodies recognize SAA from other species (see Table 1). For further information on our reagents intended for the detection of SAA in dogs, cats and horses, please refer to our Animal-SAA TechNotes or visit www.hytest.fi.

Table 1. Cross-reactivity of anti-SAA MAbs suitable for the detection of human SAA.

Cat.#	MAb	Specificity			
		Human SAA	Canine SAA	Equine SAA	Feline SAA
4SA11	VSA6	+	+	+	-
	VSA25	+	+	+	+
	SAA1	+	+	-	-
	SAA6	+	+	-	-
	SAA15	+	+	-	-
4VS4	VSA31	+	+	+	+
	VSA38	+	+	+	+
	SAA11	+	+	+	+

Development of a sandwich immunoassay for human SAA

For the development of a sandwich immunoassay for the measurement of SAA in human plasma samples, we recommend two MAb combinations: VSA25-VSA31 and VSA6-VSA38 (see Table 2). The calibration curve for the combination VSA25-VSA31 is provided in Figure 1.

Table 2. Antibody pair recommendations.

Capture MAb (Cat.# 4SA11)	Detection MAb (Cat.# 4VS4)	Limit of detection*
VSA25	VSA31	2 ng/ml
VSA6	VSA38	4 ng/ml

*In a HyTest in-house immunoassay

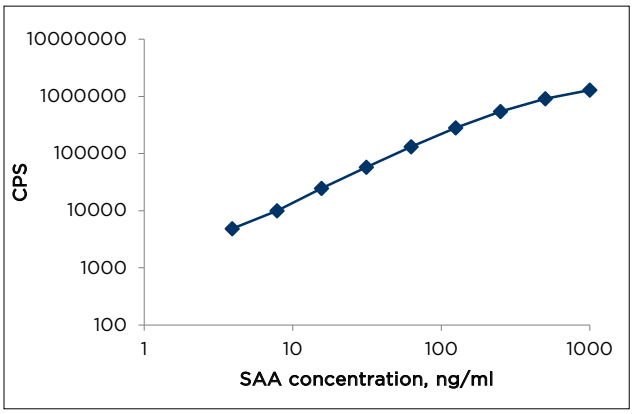

Figure 1. Calibration curve for the MAb combination VSA25-VSA31. The capture antibody VSA25 (Cat.# 4SA11) was coated onto the wells of a Costar EIA/RIA plate. The plate was blocked with a buffer that contained 1% casein and 0.05% Tween 20 at room temperature for one hour. Recombinant human SAA (Cell Sciences) and the detection MAb VSA31 (Cat.# 4VS4) labelled with europium chelate were diluted in a buffer that contained 0.05% Tween 20 and were incubated in coated plate wells for one hour at 37°C.

These assays were tested with EDTA plasma samples of healthy subjects and patients with inflammatory diseases of different origins (see Figure 2). The median plasma SAA concentration in healthy subjects, as defined by both assays, was approximately 3 µg/ml (n=18). The SAA level was considerably elevated in plasma samples of patients with an inflammatory disease. The median plasma SAA concentration in patients samples was approximately 1000 µg/ml (n=20) which is in line with literature data.

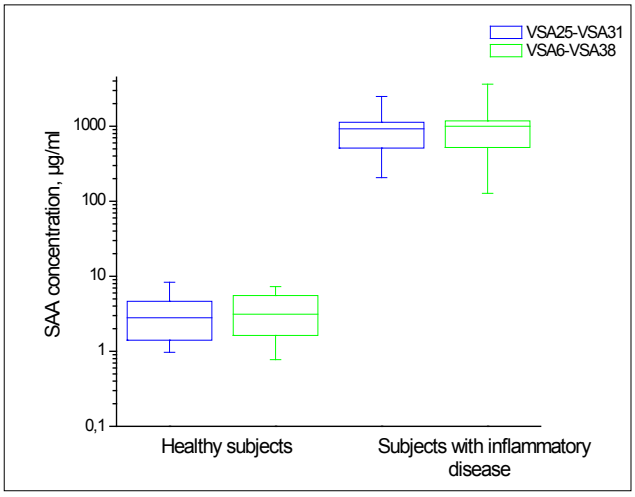

Figure 2. SAA concentration in the plasma of healthy subjects (n=18) and patients with inflammatory diseases (n=20) determined with VSA6-VSA38 and VSA25-VSA31 immunoassays. Results are displayed as a box-whisker plot. Horizontal lines indicate median values, boxes indicate values between the 25th and 75th percentiles, and whiskers indicate the minimum and maximum values.

It has been considered that endogenous SAA purified to homogeneity from human blood is not suitable as an immunoassay standard because purified SAA is recognized differently by antibodies compared to native SAA from serum (18). The difference in immunoreactivity of these two forms could be explained by the loss of native conformation of SAA during the purification process. On the other hand, the major part of SAA in serum is associated with HDL particles that can interfere with antibody binding. Therefore, acute phase HDL enriched with SAA (18) or semi-purified SAA (19) have been used as immunoassay standards.

We observed that in our immunoassays the dilution curves of recombinant human SAA (Cell Sciences) and human EDTA plasma samples with elevated SAA levels were parallel (see Figure 3). Therefore, we used the recombinant human SAA as a calibrator in our in-house immunoassays.

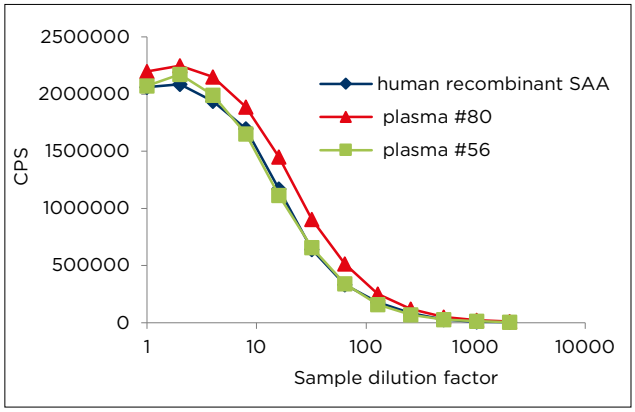

Figure 3. Titration curves of two plasma samples and recombinant human SAA obtained with the VSA25-VSA31 immunoassay. Recombinant SAA and plasma samples were serially diluted twofold. EDTA plasma samples obtained from patients with an inflammatory disease were preliminary diluted 400-fold. The initial concentration of the recombinant human SAA (Cell Sciences) was 2.5 µg/ml.

Avoiding adsorption of SAA onto microtiter plates

From earlier publications it is known that human SAA adsorbs non-specifically onto polystyrene surfaces of microtiter plates (22, 23). In our experiments we have also observed this non-specific binding. Therefore, when developing an SAA immunoassay in microtiter plates, it is important to prevent non-specific binding of SAA to the wells of a plate. The plates blocking procedure and antigen dilution buffer should be optimized to ensure that SAA non-specific binding to the plate wells is suppressed.

According to our data, casein is an effective blocking agent for SAA immunoassays (see Figure 4). For recommended MAb combinations, a buffer containing 1% casein and 0.05% Tween 20 was used for microliter plates blocking.

Please note that the suggested solid phase blocking procedure was optimized for HyTest's in-house methods. Therefore, other conditions could demonstrate a better performance in the immunoassays of our customers than those described here.

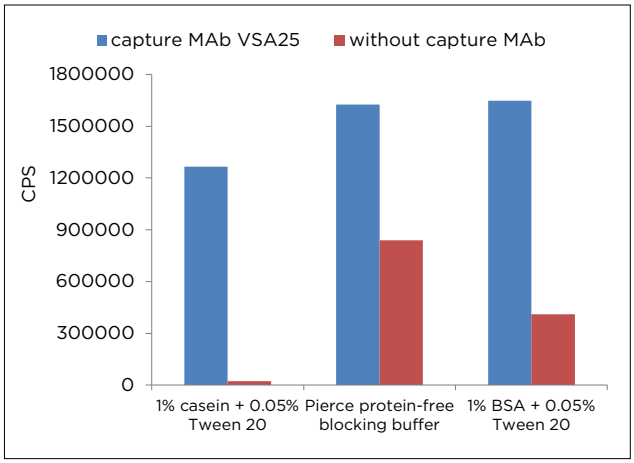

Figure 4. Comparison of the effect of three different blocking agents on the non-specific binding of SAA onto surfaces using a VSA25-VSA31 immunoassay. The capture antibody VSA25 (Cat.#4SA11) was coated onto half of the wells of a Costar EIA/RIA plate. Half of the plate wells were left uncoated. The plate wells were blocked with buffer containing (1) 1% casein (Sigma-Aldrich, # C7078) and 0.05% Tween 20, (2) Pierce Protein-Free Blocking Buffer (Thermo Scientific, # 37572), or (3) 1% bovine albumin (Calbiochem, fatty acid free, # 126575) and 0.05% Tween 20 for thirty minutes at room temperature. EDTA plasma with an elevated SAA level (2490 µg/ml) was diluted 3200-fold in a buffer containing 0.05% Tween 20 and added to the MAb-coated and uncoated plate wells simultaneously with the detection MAb VSA31 (Cat.# 4VS4) labelled with europium chelate and incubated for one hour at 37°C.

SAA immunodetection in Western blotting

All MAbs recognize both the recombinant SAA1 and endogenous SAA protein in human plasma samples in Western blotting. Staining with MAb SAA11 produced a stronger signal than other antibodies. Figure 5 illustrates the detection of endogenous SAA in the plasma sample of a patient with an inflammatory disease with the MAbs SAA1, SAA11 and SAA15.

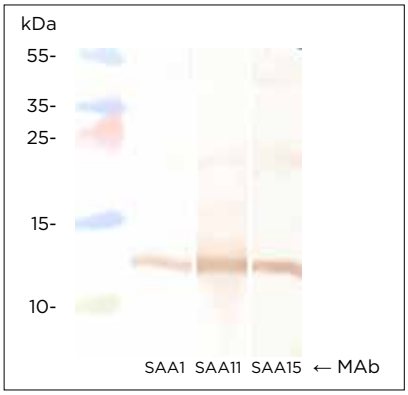

Figure 5. Immunostaining of endogenous SAA. EDTA plasma of a patient with an increased SAA level (2490 µg/ml) was analyzed by Western blotting using the MAbs SAA1 and SAA15 (Cat.# 4SA11), and SAA11 (Cat.# 4VS4). Plasma proteins were separated by using tricine-SDS electrophoresis in reducing conditions and then transferred to a nitrocellulose membrane. After blocking, the membrane was cut into strips, which were exposed to different antibodies. Molecular masses of the marker proteins are shown on the left in kDa.

Ordering information

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Serum amyloid A (SAA), human	4SA11	VSA6	IgG1	EIA, WB
		VSA25	IgG1	EIA, WB
		SAA1	IgG1	EIA, WB
		SAA6	IgG1	EIA, WB
		SAA15	IgG1	EIA, WB
Serum amyloid A (SAA), animal	4VS4	VSA31	IgG2a	EIA, WB
		VSA38	IgG2a	EIA, WB
		SAA11	IgG2b	EIA, WB

ANTIGENS

Product name	Cat. #	Purity	Source
Serum amyloid A1 (SAA1), human, recombinant	8SA1	>95%	Recombinant
Serum amyloid A2 (SAA2), human, recombinant	8SA2	>95%	Recombinant

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