Advanced ImmunoChemical Inc.
Providing for the Advancement of Medical Science

ASSAY NOTES
Product Information

Courtesy of HyTest, Ltd

Human serum amyloid A (SAA)

Reagents for the development of SAA immunoassays

Advanced ImmunoChemical 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 offer 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, 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 Assay Notes or visit www.advimmuno.com

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
2-SAA-h	VSA6	+	+	+	-
	VSA25	+	+	+	+
	SAA1	+	+	-	-
	SAA6	+	+	-	-
	SAA15	+	+	-	-
2-SAA-a	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, two MAb combinations are recommended: 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.# 2-SAA-h)	Detection MAb (Cat.# 2-SAA-a)	Limit of detection*
VSA25	VSA31	2 ng/ml
VSA6	VSA38	4 ng/ml

*In a AdvImmuno in-house immunoassay.

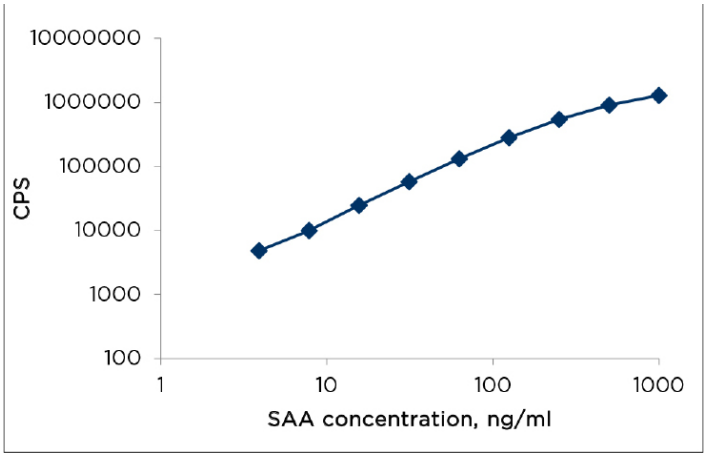

Figure 1.Calibration curve for the MAb combination VSA25-VSA31. *The capture antibody VSA25 (Cat.# 2-SAA-h) was coated onto the wells of a Costar EIA/RIA plate. The plate was blocked with a buffer that contained 1% casein and 0.05% Tween 20 at room temperature for one hour. Recombinant human SAA (Cell Sciences) and the detection MAb VSA31 (Cat.# 2-SAA-a) 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.

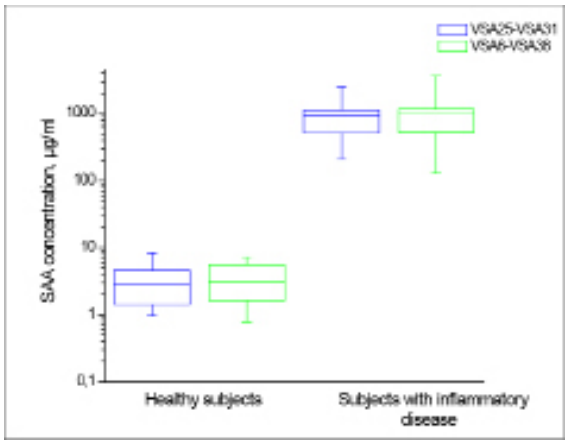

Figure 2. SAA concentration in the plasma of healthy subjects (n=18) and patients with inflammatory diseases (n=20) determined with VSA6-VSA38 and VSA25-VSA31 immunoassays. *Results are displayed as a box-whisker plot. Horizontal lines indicate median values, boxes indicate values between the 25th and 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 (1). 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 (1) or semi-purified SAA (2) have been used as immunoassay standards.

The dilution curves of recombinant human SAA (Cell Sciences) and human EDTA plasma samples with elevated SAA levels were parallel (see Figure 3). Therefore, recombinant human SAA can be used as a calibrator for immunoassays.

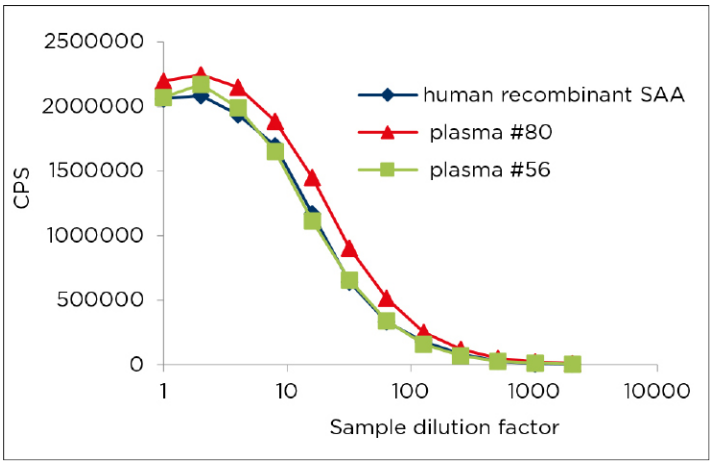

Figure 3. Titration curves of two plasma samples and recombinant human SAA obtained with the VSA25-VSA31 immunoassay. *Recombinant SAA and plasma samples were serially diluted twofold. EDTA plasma samples obtained from patients with an inflammatory disease were preliminary diluted 400-fold. The initial 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 (3, 4). 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 data shown below, 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 plate blocking.

Please note: other conditions could demonstrate a better performance in the immunoassays of customers than those described here.

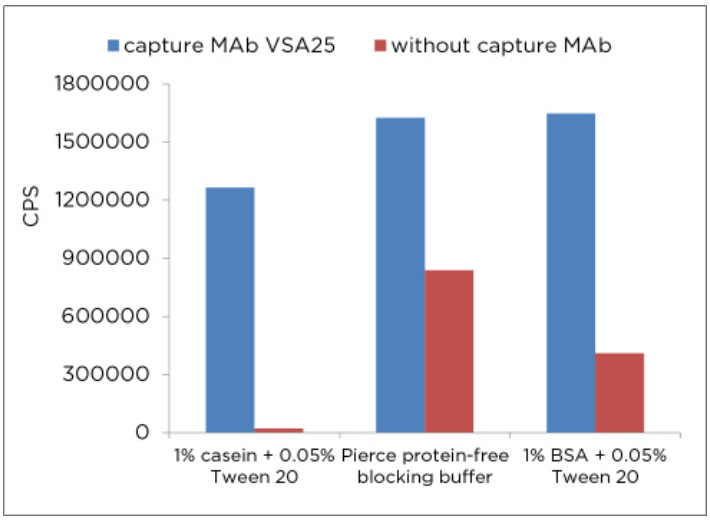

Figure 4. Comparison of the effect of three different blocking agents on the non-specific binding of SAA onto surfaces using a VSA25-VSA31 immunoassay. *The capture antibody VSA25 (Cat.#2-SAA-h) was coated onto half of the wells of a Costar EIA/RIA plate. Half of the plate wells were left uncoated. The 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.# 2-SAA-a) 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.

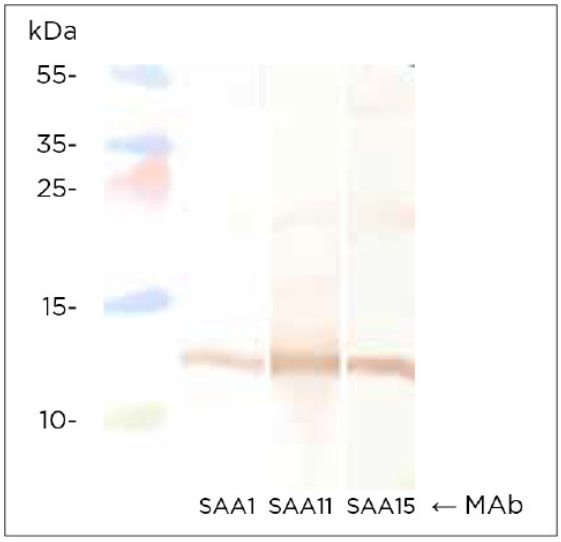

Figure 5. Immunostaining of endogenous SAA. *EDTA plasma of a patient with an increased SAA level (2490 µg/ml) was analyzed by Western blotting using the MAbs SAA1 and SAA15 (Cat.# 2-SAA-h), and SAA11 (Cat.# 2-SAA-a). Plasma proteins were separated by using tricine- SDS electrophoresis in reducing conditions 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	2-SAA-h	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	2-SAA-a	VSA31	IgG2a	EIA, WB
		VSA38	IgG2a	EIA, WB
		SAA11	IgG2b	EIA, WB

References

1. Godenir N et al. J. Immunol. Methods. 1985, 83: 217-225.

2. McDonald TL et al. J. Immunol. Methods. 1991, 144(2):149-155.

3. Marhaug G. A. Scand. J. Immunol. 1983, 18: 329-338.

4. Časl MT and Grubb A. Ann. Clin. Biochem. 1993, 30: 278-286.

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