Human serum amyloid A (SAA)

The serum amyloid A apolipoprotein family consists of three members that are coded by different genes in human beings: SAA1, SAA2, and SAA4 (1-3). SAA1 and SAA2 are so-called acute phase isoforms and 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. Meanwhile, SAA4 consists of 112 amino acid residues, and the homology between SAA4 and acute-phase SAA isoforms is approximately 50%.

The crystal structure of human recombinant SAA1 containing an N-terminal histidine tag was recently published. It was reported that SAA contains four α-helix regions that span the amino acid residues (a.a.r.) 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 and it has been suggested that SAA is involved in the recycling of cholesterol from damaged tissues. It is considered to 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 have demonstrated 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.

CLINICAL UTILITY
- **Inflammation**
- **Tissue injury**

SAA as a diagnostic marker

SAA is a non-specific marker of inflammation, the concentration of which in the blood increases in response to inflammatory stimuli such as tissue injury, infection, or trauma. Similarly, to the 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 the concentration of SAA then 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 several 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).

MONOCLONAL ANTIBODIES SPECIFIC TO HUMAN SAA

Hytest offers a set of monoclonal anti-SAA antibodies that are suitable for the development of sandwich immunoassays for the quantitative detection of human SAA in blood samples. A subset of them also recognizes SAA from other species (Table 1). For further information on our reagents that are intended for the detection of SAA in cats, horses, and dogs, please refer to our animal SAA TechNotes or visit www.hytest.fi.

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 several pair combinations (Table 2). Two of the capture-detection pair recommendations A496-A491 and A491-A496 can be used for SAA detection in lateral flow platforms as well. All of the recommended MAb combinations recognize recombinant SAA1 (Cat.# 8SA1), SAA2 (Cat.# 8SA2), and SAA from human blood.

These assays were tested with EDTA plasma samples of healthy subjects and patients with inflammatory diseases of different origins (Figure 1). The SAA level was considerably elevated in plasma samples of patients with inflammatory disease.

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	A491	+	+	+	+
	A496	+	-	-	-
	VSA6	+	+	+	-
	VSA25	+	+	+	+
	SAA1cc	+	+	-	-
	SAA6	+	+	-	-
	SAA15cc	+	+	-	-
4VS4	SAA19cc	+	+	+	+
	SAA21cc	+	+	+	+
	VSA38cc	+	+	+	+
	VSA31cc	+	+	+	+

Table 2.
Human SAA antibody pair recommendations.

Capture	Detection
A496	A491
A496	SAA19cc
SAA19cc	A496
A496	SAA21cc

Cross-reactivity with human SAA variants

We have tested the cross-reactivity of our recommended antibody pairs with the most common human SAA allelic variants including SAA1.1, SAA1.3, SAA1.5, SAA2.1, and SAA2.2 (Figure 2). Recombinant SAA proteins were expressed in *E. coli* and purified under identical conditions. The prototype immunoassays using antibody pairs A496-A491, A496-SAA19cc, A496-SAA21cc, and SAA19cc-A496 detected all of the tested human SAA variants (Figure 3).

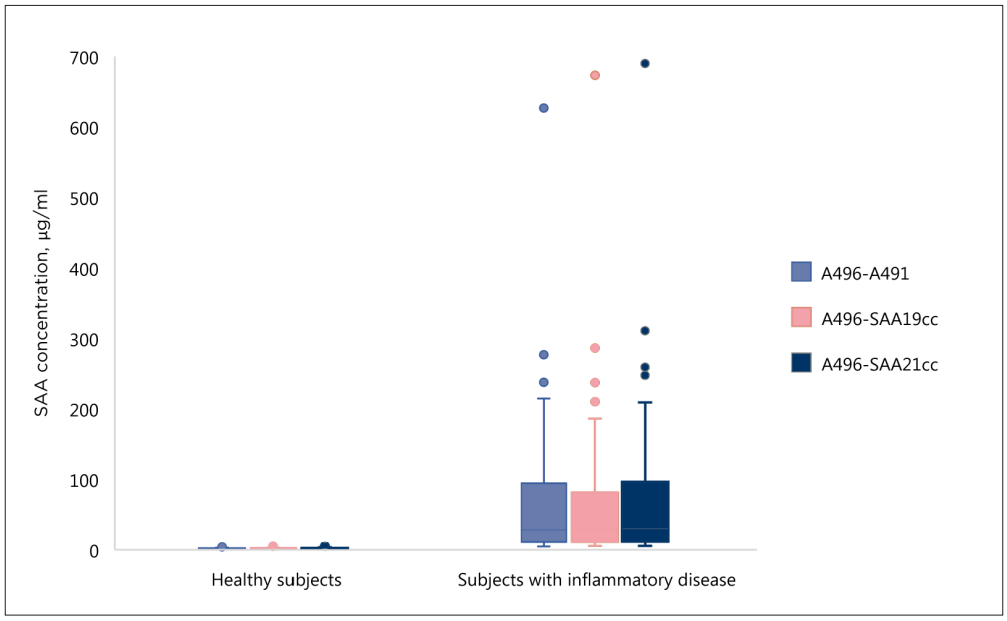

Figure 1.
SAA concentration in the plasma of healthy subjects (n=18) and patients with inflammatory disease (n=28) determined with in-house immunoassays. The results are displayed as a box-whisker plot.

a.a.r.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Human SAA1.1	R	S	F	F	S	F	L	G	E	A	F	D	G	A	R	D	M	W	R	A	Y	S	D	M	R	E	A	N	Y	I	G	S	D	K	Y	F	H
Human SAA1.3	R	S	F	F	S	F	L	G	E	A	F	D	G	A	R	D	M	W	R	A	Y	S	D	M	R	E	A	N	Y	I	G	S	D	K	Y	F	H
Human SAA1.5	R	S	F	F	S	F	L	G	E	A	F	D	G	A	R	D	M	W	R	A	Y	S	D	M	R	E	A	N	Y	I	G	S	D	K	Y	F	H
Human SAA2.1	R	S	F	F	S	F	L	G	E	A	F	D	G	A	R	D	M	W	R	A	Y	S	D	M	R	E	A	N	Y	I	G	S	D	K	Y	F	H
Human SAA2.2	R	S	F	F	S	F	L	G	E	A	F	D	G	A	R	D	M	W	R	A	Y	S	D	M	R	E	A	N	Y	I	G	S	D	K	Y	F	H
a.a.r.	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74
Human SAA1.1	A	R	G	N	Y	D	A	A	K	R	G	P	G	G	V	W	A	A	E	A	I	S	D	A	R	E	N	I	Q	R	F	F	G	H	G	A	E
Human SAA1.3	A	R	G	N	Y	D	A	A	K	R	G	P	G	G	A	W	A	A	E	A	I	S	D	A	R	E	N	I	Q	R	F	F	G	H	G	A	E
Human SAA1.5	A	R	G	N	Y	D	A	A	K	R	G	P	G	G	A	W	A	A	E	V	I	S	D	A	R	E	N	I	Q	R	F	F	G	H	G	A	E
Human SAA2.1	A	R	G	N	Y	D	A	A	K	R	G	P	G	G	A	W	A	A	E	V	I	S	N	A	R	E	N	I	Q	R	L	T	G	H	G	A	E
Human SAA2.2	A	R	G	N	Y	D	A	A	K	R	G	P	G	G	A	W	A	A	E	V	I	S	N	A	R	E	N	I	Q	R	L	T	G	R	G	A	E
a.a.r.	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104							
Human SAA1.1	D	S	L	A	D	Q	A	A	N	E	W	G	R	S	G	K	D	P	N	H	F	R	P	A	G	L	P	E	K	Y							
Human SAA1.3	D	S	L	A	D	Q	A	A	N	E	W	G	R	S	G	K	D	P	N	H	F	R	P	A	G	L	P	E	K	Y							
Human SAA1.5	D	S	L	A	D	Q	A	A	N	E	W	G	R	S	G	K	D	P	N	H	F	R	P	A	G	L	P	E	K	Y							
Human SAA2.1	D	S	L	A	D	Q	A	A	N	K	W	G	R	S	G	R	D	P	N	H	F	R	P	A	G	L	P	E	K	Y							
Human SAA2.2	D	S	L	A	D	Q	A	A	N	K	W	G	R	S	G	R	D	P	N	H	F	R	P	A	G	L	P	E	K	Y							

Figure 2.
Sequence alignment of human SAA proteins used for the cross-reactivity study. Amino acid residues substituted among variants are highlighted in blue. The nomenclature of SAA variants is based on the recommendations provided by Sipe (18).

Recombinant SAA4 protein was also expressed in *E. coli* and purified to homogeneity. The recommended antibody pairs do not recognize a constitutive isoform SAA4 (Figure 4).

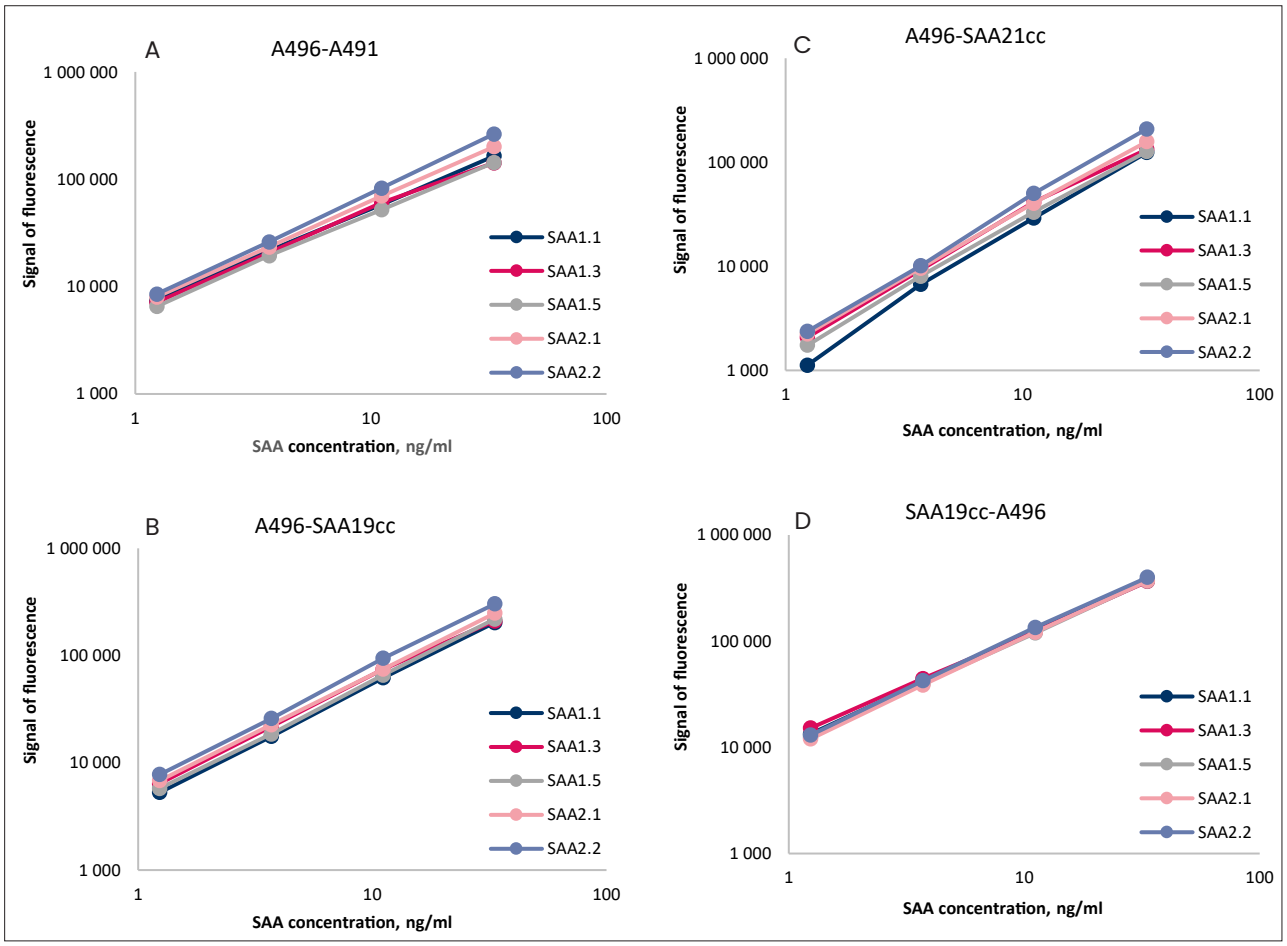

Figure 3.
Calibration curves for the assays using A) A496-A491, B) A496-SAA19cc, C) A496-SAA21cc, and D) SAA19cc-A496 antibody pairs.
Recombinant SAA variants were used as calibrators.

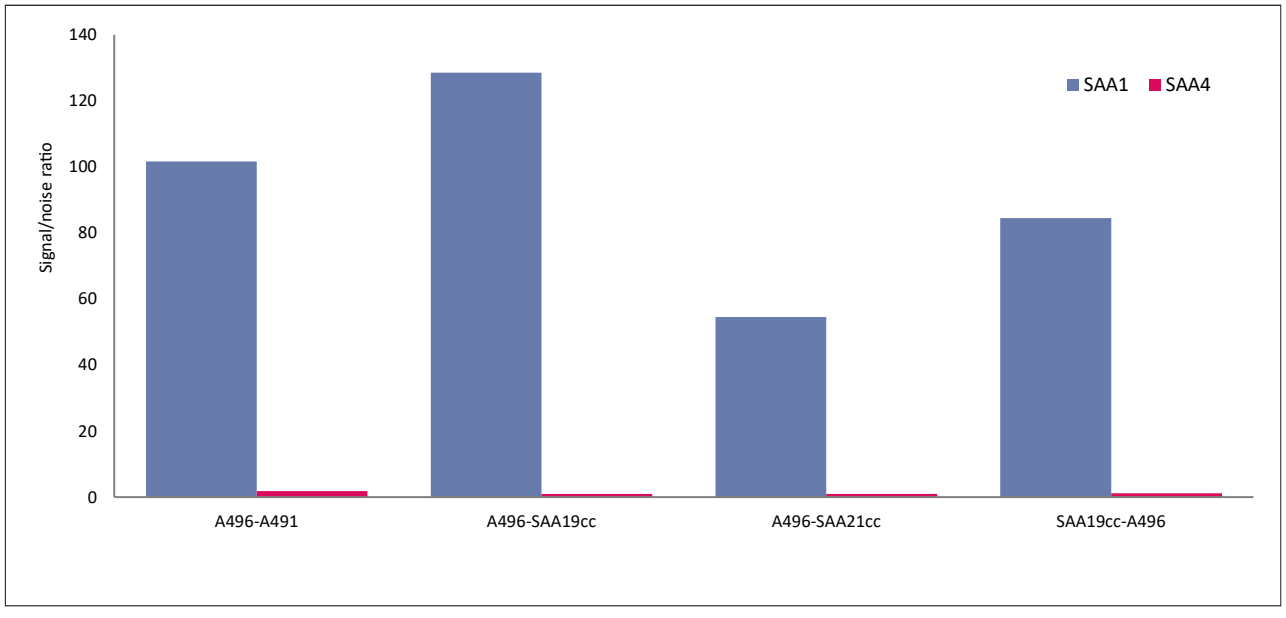

Figure 4.
Comparison of SAA1 and SAA4 immunoreactivity for the assays using Hytest's antibody pairs. SAA1 and SAA4 concentrations were 33 ng/ml.

RECOMBINANT HUMAN SAA

Hytest provides recombinant human SAA1 (variant SAA1.1) and SAA2 (variant SAA2.1) expressed in *E. coli*. Amino acid sequences of recombinant SAA1.1 and SAA2.1 are provided in Figure 2. Both proteins contain an additional N-terminal methionine residue. The purity of recombinant SAA proteins exceeds 95% (Figure 5).

Both proteins demonstrated similar immunochemical activity (Figure 6A). In assays using recommended antibody pairs, the dilution curves of recombinant human SAA proteins and human EDTA plasma samples were parallel (Figure 6B). Therefore, SAA concentration can be accurately determined in diluted plasma samples using purified recombinant SAA as a calibrator.

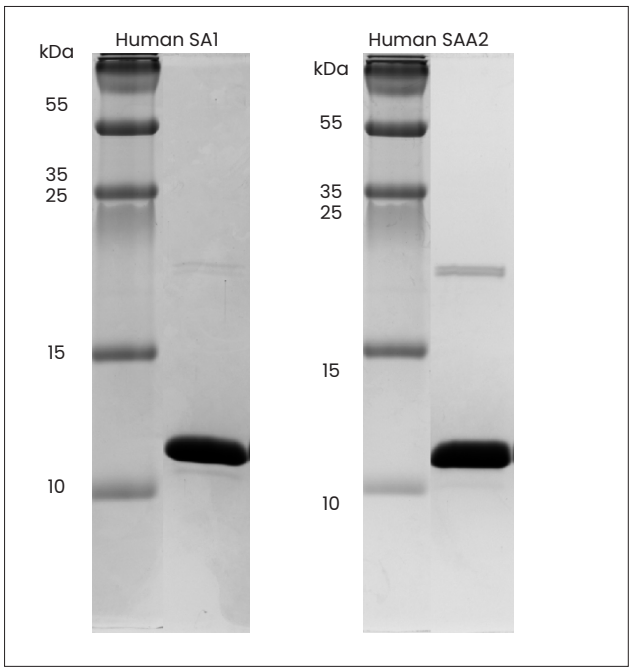

Figure 5.
Tricine-SDS-PAGE of recombinant human SAA proteins in reducing conditions. 5 µg of protein was loaded for each lane. Gels were stained using Coomassie Brilliant Blue R-250.

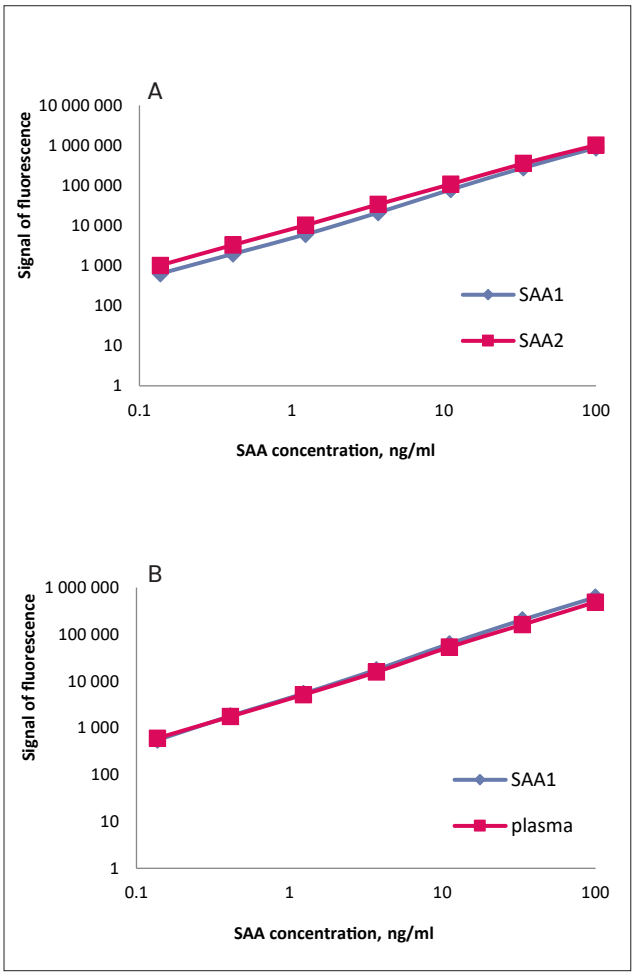

Figure 6.
Calibration curves for the human SAA prototype assay using the antibody pair A496-SAA19cc. A) human recombinant SAA1 and SAA2 were used as the antigens. B) human recombinant SAA1 and EDTA plasma samples were used as the antigens.

Avoiding adsorption of SAA onto microtiter plates

From earlier publications, it is acknowledged that human SAA adsorbs non-specifically onto polystyrene surfaces of microtiter plates (19, 20). The plates blocking procedure and antigen dilution buffer might require optimization 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 7). Blocking buffer containing 1% casein and 0.05% Tween 20 is suggested for plates blocking.

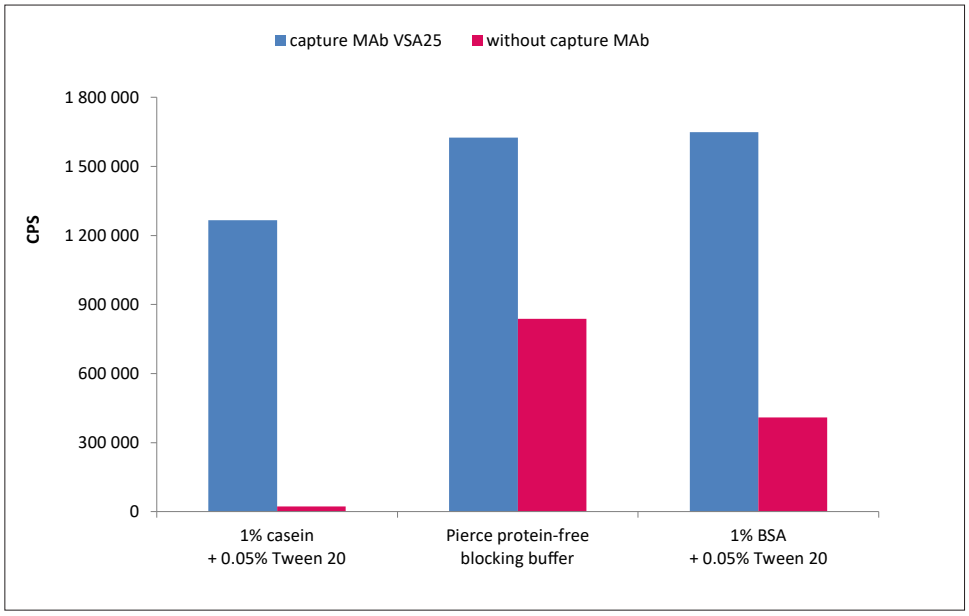

Figure 7.

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 one-half of the wells of a Costar EIA/RIA plate. Meanwhile, the other half of the plate wells were left uncoated. The plate wells were blocked with a buffer containing (1) 1% casein and 0.05% Tween 20, (2) Pierce Protein-Free Blocking Buffer, or (3) 1% bovine albumin and 0.05% Tween 20 for thirty minutes at room temperature. EDTA plasma with an elevated SAA level (2,490 µg/ml) was diluted 3,200-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.

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ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Serum amyloid A (SAA), human	4SA11	A491	IgG2b	*In vitro*, EIA, rat monoclonal antibody
		A496	IgG1	*In vitro*, EIA, rat monoclonal antibody
		SAA1cc	IgG1	*In vitro*, EIA, WB
		SAA6	IgG1	EIA, WB
		SAA15cc	IgG1	*In vitro*, EIA, WB
		VSA6	IgG1	EIA, WB
		VSA25	IgG1	EIA, WB
Serum amyloid A (SAA), animal	4VS4	SAA19cc	IgG2a	*In vitro*, EIA
		SAA21cc	IgG2b	*In vitro*, EIA
		VSA31cc	IgG2a	*In vitro*, EIA, WB
		VSA38cc	IgG2a	*In vitro*, 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

Please note that some or all data presented in this TechNotes has been prepared using MAbs produced *in vivo*. MAbs produced *in vitro* are expected to have similar performance.

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