

# Feline, canine and equine serum amyloid A (SAA)

Serum amyloid A (SAA) is one of the major acute phase proteins in many species including cats, dogs and horses. SAA is secreted by the liver into plasma and is also produced by various extrahepatic tissues at the inflammation sites. Most circulating SAA binds to plasma high-density lipoproteins (HDL). Similarly to other acute phase reactants, SAA concentration increases in response to inflammatory stimuli.

## SAA as a diagnostic marker

SAA is a sensitive marker of inflammation and tissue damage. In veterinary medicine, SAA measurements in blood might be used for the diagnosis of subclinical inflammation, the monitoring of treatment efficacy in animals with infections or inflammatory conditions, and the monitoring of patients who are undergoing surgery.

## Biochemical properties of SAA

Feline and canine SAA consist of 111 a.a.r. while equine SAA consists of 110 a.a.r. Feline, canine and equine SAA contain an insertion of eight amino acids in the central part of the molecule as compared to human or mouse SAA. The primary amino acid sequences of SAA from a variety of mammalian species are highly conserved and share 61-80% in sequence identities (Lu et al., 2014). It suggests that all SAA family members share a common structural fold.

X-ray crystal structures of lipid-free human SAA1 and murine SAA3 show that SAA monomer forms a four-helix bundle structure with ~75%  $\alpha$ -helical content (Lu et al., 2014; Derebe et al., 2014; Hu et al., 2019). However, in solution the  $\alpha$ -helical content of lipid-free SAA has been shown to be lower. Murine lipid-free SAA1 was shown to be ~25%  $\alpha$ -helical at 5°C, largely

unfolded at 25°C (<10%  $\alpha$ -helix) and fully unfolded at 37°C (Frame et al., 2020; Jayaraman et al., 2015). In other words, in aqueous solution under near-physiological conditions free SAA lacks stable secondary structure. Upon binding to lipids, SAA1 acquired  $\alpha$ -helical structure (~50%) and its conformation remained invariant upon heating from 5 to 25°C (Frame et al., 2020). In free SAA1 in solution at 5°C, most helices were located in the region 1-70 a.a.r. Unlike the N-terminal region, most of the C-terminal third of SAA lacks a stable secondary structure free in solution and in model SAA-containing lipoproteins.

Furthermore, analysis of human and murine SAA proteins in solution has revealed the presence of various oligomeric forms. Different SAA proteins form oligomers of varying composition. It was suggested that SAA proteins may be unstable as free monomers in solution, and that other hydrophobic interactions may be needed to bury their exposed hydrophobic surfaces. SAA oligomers contain a hydrophobic cavity that has been shown to bind retinol (Hu et al., 2019). Using mice as a model, it has been demonstrated that although the majority of circulating SAA is associated with HDL, a fraction of SAA circulates freely in the bloodstream after acute infection. Freely circulating SAA bound the majority of retinol in the bloodstream of mice with acute infection.

## CLINICAL UTILITY

- Sensitive marker of inflammation
- Monitoring of treatment

## Reagents for the development of reliable, species-specific SAA immunoassays

At Hytest, we provide several monoclonal antibodies that can be used for the development of immunoassays that enable the detection of feline, canine and equine SAA. Antibodies recognize SAA from animal blood samples. Furthermore, we also offer recombinant feline, canine and equine SAA proteins.

### Monoclonal antibodies specific to SAA

Hytest offers a set of monoclonal anti-SAA antibodies that are suitable for the development of sandwich immunoassays for the quantitative detection of feline, canine and equine SAA in blood samples. A subset of them also recognize human SAA1 (Table 1).

## FELINE SAA

### Isoform composition of SAA protein in individual cats

SAA genes are poorly characterized in cats. One study reported about existence of up to four functional genes coding SAA proteins per individual cat (van Rossum et al., 2004). There are several polymorphisms in feline SAA gene, which lead to the replacement of one or a few amino acids in the protein. The most commonly reported substitutions were E1/Q1 (Tei et al., 2018), I29/K29 (van Rossum et al., 2004), Q45/R45 (Niewold et al., 1999; Tei et al., 2018; van Rossum et al., 2004), A51/V51 (Niewold et al., 1999; Tei et al., 2018; van Rossum et al., 2004), N75/S75 (van Rossum et al., 2004). Several additional substitutions in the C-terminus of feline SAA (E88/A88, Y102/F102, E105/A105, D109/S109) were encountered in feline SAA sequences found in sequence databases (for example, UniProtKB accession number P19707). Thus, genome of cats may contain different combinations of saa gene variants, as was shown for Abyssinian, Siamese, domestic shorthair cat, and Japanese domestic cat breeds (Niewold et al., 1999; Tei et al., 2018; van Rossum et al., 2004). At present, there is no data about allele frequencies of SAA genes in cats.

### Clinical significance of feline SAA

SAA is a sensitive non-specific marker of acute and chronic inflammatory conditions such as infection, tissue injury, trauma, surgery, neoplasia or immunological disorders. SAA levels can be of assistance in disease diagnosis and management when interpreted in conjunction with clinical and laboratory parameters. SAA concentration was markedly increased 8 hours and reached a maximum 24-48 hours after induction of inflammation in cats (Kajikawa et al., 1999). SAA concentration increased as soon as 3-6 hours in response to surgery reaching the peak values at 21-24 hours following the surgery (Sasaki et al., 2003). Thus, feline SAA is an acute phase reactant at the early stage of inflammation.

Possibly due to the use of different methodologies, SAA concentrations determined in healthy cats differ from study to study. The concentrations of SAA in the normal feline serum samples were (mean  $\pm$  SD)  $16.6 \pm 11.4$   $\mu\text{g/ml}$  (Kajikawa et al., 1999),  $0.6 \pm 1.06$   $\mu\text{g/ml}$  (Sasaki et al., 2003), and  $10.21 \pm 8.32$   $\mu\text{g/ml}$  (Giordano et al., 2004).

During inflammation SAA concentration may increase up to 100-fold and even higher in cats, and elevated SAA concentration was reported in different inflammation-associated pathological conditions in cats (Table 2). The acute phase response usually lasts for several days after which the concentration of SAA gradually decreases in the absence of a new stimulus. Using time-course monitoring of SAA concentrations in a cat with pancreatitis, it was shown that SAA concentration increased at the onset of the disease and decreased

**Table 1.**  
Specificities of anti-SAA MAbs.

MAb	Feline SAA	Canine SAA	Equine SAA	Human SAA1
F501	+	-	-	-
F529	+	-	-	-
F550	+	-	-	-
F571	+	-	-	-
F173	+	+	+	+
F227	+	+	+	+
F231	+	+	+	+
F240	+	+	+	+
SAA19cc	+	+	+	+
SAA21cc	+	+	+	+
VSA31cc	+	+	+	+
VSA34cc	+	+	+	+
VSA38cc	+	+	+	+
VSA2	-	+	+	+
VSA43	+/-	+	+	+

in response to treatment with an improvement in the clinical condition (Tamamoto et al., 2009). These findings suggest that serial measurements of SAA concentrations were useful for evaluating response to treatment and disease exacerbation in a cat with pancreatitis. A recent study demonstrated that high-sensitive SAA assay reflect low grade inflammation in cats with obesity disease. Elevated level of SAA in conjunction with low adiponectin concentration and/or hyperlipidemia can classify overweight cats as having obesity disease (Okada et al., 2019).

It has also been suggested that SAA might be used as a prognostic biomarker. SAA concentration at the time of first examination at the hospital was demonstrated as a significant prognostic factor in cats with neoplastic diseases, inflammatory diseases, and other diseases including renal failure, diabetes mellitus, hyperthyroidism, cardiomyopathy, etc. Median survival time for cats with elevated levels of SAA was much shorter (72 days) compared with cats suffering from these diseases with non-elevated SAA level (571 days) (Tamamoto et al., 2013).

## Development of a sandwich immunoassay for feline SAA

For the development of a sandwich immunoassay for the measurement of SAA in blood samples, we recommend several MAb combinations (Table 3).

**Table 2.**  
SAA concentrations in cats with various diseases (Yuki et al., 2020).

Diagnosis	# of cats	Median SAA conc., µg/ml	SAA conc., interquartile range, µg/ml
Upper respiratory tract infections	41	141.1	17.9–155.4
Pneumonia	14	134.3	12.5–168.7
Gingivostomatitis	37	1.3	0–52.8
Gastroenteritis	59	0.3	0–29.0
Pancreatitis	20	3.9	0–138.1
Hepatitis/cholangitis	8	12	0–123.5
Chronic kidney disease	83	0.03	0–5.9
Lower urinary tract diseases	51	0	0–53.8
Pyometra	7	154.8	0.1–182.4
Ketoacidosis	8	4.1	0.3–60.5
Feline infectious peritonitis	5	143.9	69.8–147.5
Traumatic diseases	35	123.9	18.8–152.3
Solid tumor	19	0.3	0–10.7
Round cell tumor	30	0.5	0–42.7
Cardiomyopathy	9	0	0–2.9
Hyperthyroidism	13	0	0–20.4
Diabetes mellitus	5	0	0.08

**Table 3.**  
Antibody pair recommendations for quantitative feline SAA sandwich immunoassay with recommended incubation temperatures.

Capture MAb	Detection MAb	Recommended incubation temperature
<b>F227</b>	<b>F529</b>	<b>RT</b>
<b>F231</b>	<b>F550</b>	<b>RT</b>
F240	F501	RT
F240	F550	RT
SAA19cc	VSA34cc	RT
F571	F173	37°C*

\*Antibody pair F571-F173 poorly recognizes SAA at room temperature. With this pair immunoassays should be carried out at 37°C.

**Table 4.**  
Antibody pair recommendations for detecting feline SAA in lateral flow.

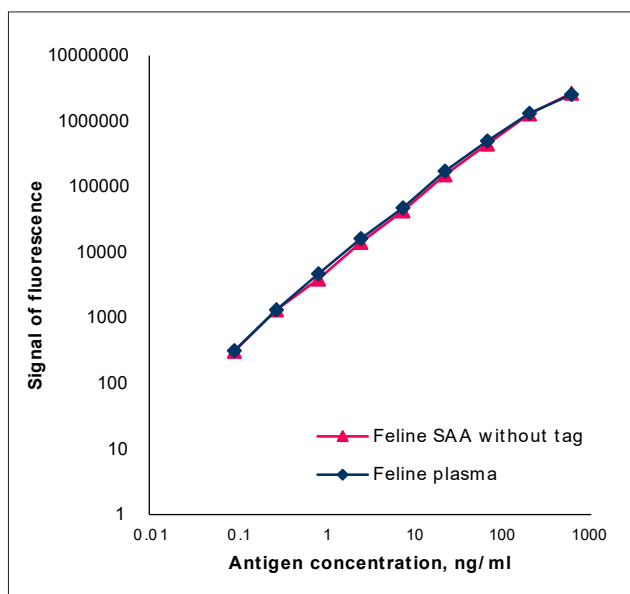
Capture MAb	Detection MAb
F550	F231
F529	F227
F501	F240

## Measurement of SAA concentrations in feline plasma samples

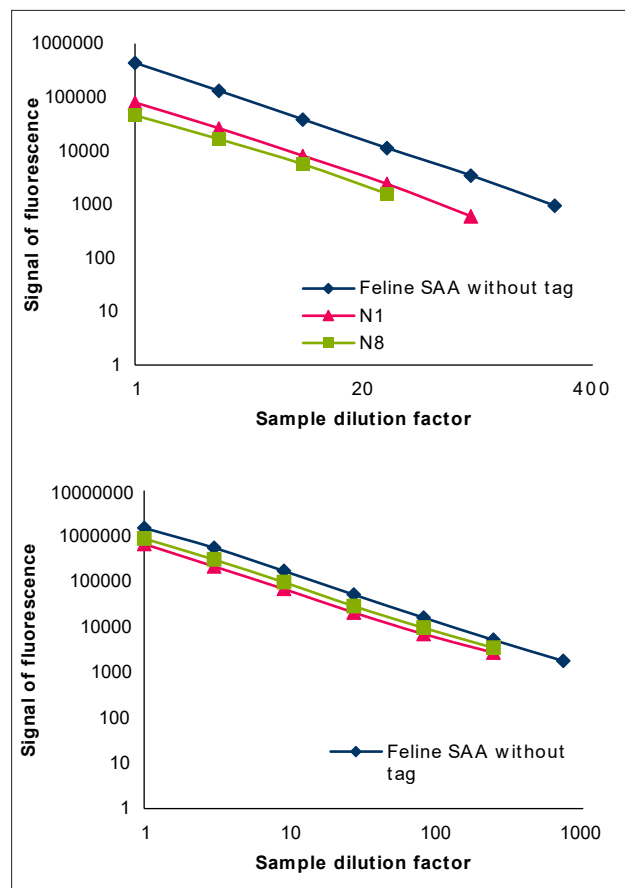
The dilution curves of recombinant feline SAA proteins (catalog # 8FS5, 8FT7) and feline plasma samples were parallel for all recommended antibody combinations except for SAA19cc-VSA34cc. Figure 1 illustrates representative dilution curves of pooled feline EDTA plasma and recombinant feline SAA for the prototype assay using pair of antibodies F231-F550. Dilution curves of individual samples from healthy cats and cats with inflammation induced by surgery for the prototype assay using pair of antibodies F227-F529 are provided in Figures 2A and 2B, correspondingly.

For immunoassays in which the dilution curves of plasma and recombinant feline SAA are parallel, SAA concentration can be accurately determined in diluted plasma samples using purified recombinant SAA as a calibrator. For antibody pair SAA19cc-VSA34cc we recommend using recombinant SAA with tag (Cat.# 8FS5) as a control in immunoassays, for all other pairs both recombinant SAA antigens could be used as calibrators.

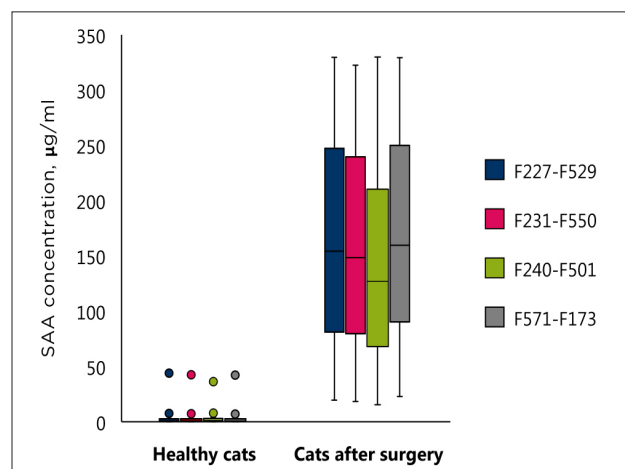
Prototype immunoassays were tested with EDTA plasma samples of healthy cats (n=22) and cats with inflammation induced by surgery (n=21). Elevated SAA levels were observed in cats with inflammation compared to healthy cats (Figure 3).



**Figure 1.** Calibration curves for the feline SAA prototype assay using pair of antibodies F231-F550 and feline SAA without tag (catalog # 8FT7) and pooled feline EDTA plasma as the antigens. SAA concentration in pooled plasma was 133  $\mu\text{g}/\text{ml}$ . Plasma was diluted 219.5-fold prior to assay.



**Figure 2.** Dilution curves for the feline SAA prototype assay using pair of antibodies F227-F529 and samples of plasma as the antigens. (A) Healthy cats (N1, N8). SAA concentration in plasma samples was 0.55  $\mu\text{g}/\text{ml}$  and 0.35  $\mu\text{g}/\text{ml}$  for N1 and N8 samples, respectively. Plasma samples were diluted 35-fold prior to assay. The initial concentration of the recombinant feline SAA without tag (catalog # 8FT7) was 72 ng/ml. (B) Cats after surgery (A26, A30). SAA concentration in plasma samples was 178  $\mu\text{g}/\text{ml}$  and 217  $\mu\text{g}/\text{ml}$  for A26 and A30 samples, respectively. Plasma samples were diluted 2000-fold prior to assay. The initial concentration of the recombinant feline SAA without tag was 200 ng/ml (catalog # 8FT7).



**Figure 3.** SAA concentrations in plasma samples of healthy cats and cats after surgery. Plasma samples from healthy cats were diluted in the range of 100-2500-fold before testing. Plasma samples from cats after surgery were diluted 8000-fold before testing.

## Cross-reactivity with feline SAA variants

We investigated the cross-reactivity of our key recommended antibody pairs with the most common feline SAA variants including SAA Q9XSG7 (UniProtKB accession number Q9XSG7, used as a reference sequence), four variants of SAA Q9XSG7 containing single amino acid substitutions I29/K29, Q45/R45, A51/V51, N75/S75, and SAA P19707 (UniProtKB accession number P19707), which has five amino acid residues that are different from those of SAA Q9XSG7 (Figure 4).

Prototype immunoassays using F227-F529, F231-F550, F240-F550 antibody pairs detected all tested feline SAA variants. There were low detection levels of SAA N75/S75 variant for prototype immunoassay using F240-F501 antibody pair, and SAA P19707 variant for prototype immunoassay using F571-F173 antibody pair. At the same time, these two prototype immunoassays detected all other feline SAA variants.

MAbs SAA19cc and SAA21cc poorly recognized I29/K29 variant compared to other variants. MAb VSA34cc recognized all tested variants.

There are no data in the literature on the allele frequency of SAA genes in cats, so it is difficult to say what is the significance of weak recognition of one of the variants by antibodies on accurate determination of the SAA level in the blood and detecting inflammation.

## Recombinant feline SAA

Hyttest provides two versions of recombinant feline SAA with identical sequences corresponding to UniprotKB accession number Q9XSG7. One version contains an additional 10 a.a. affinity tag on the N-terminus of the SAA molecule (Cat.# 8FS5). A non-tagged feline SAA contains only one additional N-terminal methionine residue (catalog # 8FT7). The purity of recombinant feline SAA proteins exceeds 95% (Figure 5). Both proteins demonstrate similar immunochemical activity (Figure 6).

Amino acid residues	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
Feline SAA (UniProtKB Q9XSG7)	E	W	Y	S	F	L	G	E	A	A	Q	G	A	W	D	M	W	R	A	Y	S	D	M	R	E	A	N	Y	I	G	A	D	K	Y	F	H	A
Feline SAA (UniProtKB Q9XSG7) I29/K29	E	W	Y	S	F	L	G	E	A	A	Q	G	A	W	D	M	W	R	A	Y	S	D	M	R	E	A	N	Y	K	G	A	D	K	Y	F	H	A
Feline SAA (UniProtKB Q9XSG7) Q45/R45	E	W	Y	S	F	L	G	E	A	A	Q	G	A	W	D	M	W	R	A	Y	S	D	M	R	E	A	N	Y	I	G	A	D	K	Y	F	H	A
Feline SAA (UniProtKB Q9XSG7) A51/V51	E	W	Y	S	F	L	G	E	A	A	Q	G	A	W	D	M	W	R	A	Y	S	D	M	R	E	A	N	Y	I	G	A	D	K	Y	F	H	A
Feline SAA (UniProtKB Q9XSG7) N75/S75	E	W	Y	S	F	L	G	E	A	A	Q	G	A	W	D	M	W	R	A	Y	S	D	M	R	E	A	N	Y	I	G	A	D	K	Y	F	H	A
Feline SAA (UniProtKB P19707)	Q	W	Y	S	F	L	G	E	A	A	Q	G	A	W	D	M	W	R	A	Y	S	D	M	R	E	A	N	Y	I	G	A	D	K	Y	F	H	A
Amino acid residues	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
Feline SAA (UniProtKB Q9XSG7)	R	G	N	Y	D	A	A	Q	R	G	P	G	G	A	W	A	A	K	V	I	S	D	A	R	E	N	S	Q	R	V	T	D	F	F	R	H	G
Feline SAA (UniProtKB Q9XSG7) I29/K29	R	G	N	Y	D	A	A	Q	R	G	P	G	G	A	W	A	A	K	V	I	S	D	A	R	E	N	S	Q	R	V	T	D	F	F	R	H	G
Feline SAA (UniProtKB Q9XSG7) Q45/R45	R	G	N	Y	D	A	A	R	R	G	P	G	G	A	W	A	A	K	V	I	S	D	A	R	E	N	S	Q	R	V	T	D	F	F	R	H	G
Feline SAA (UniProtKB Q9XSG7) A51/V51	R	G	N	Y	D	A	A	Q	R	G	P	G	G	V	W	A	A	K	V	I	S	D	A	R	E	N	S	Q	R	V	T	D	F	F	R	H	G
Feline SAA (UniProtKB Q9XSG7) N75/S75	R	G	N	Y	D	A	A	Q	R	G	P	G	G	A	W	A	A	K	V	I	S	D	A	R	E	N	S	Q	R	V	T	D	F	F	R	H	G
Feline SAA (UniProtKB P19707)	R	G	N	Y	D	A	A	Q	R	G	P	G	G	A	W	A	A	K	V	I	S	D	A	R	E	N	S	Q	R	V	T	D	F	F	R	H	G
Amino acid residues	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	105	106	107	108	109	110	111
Feline SAA (UniProtKB Q9XSG7)	N	S	G	H	G	A	E	D	S	K	A	D	Q	E	A	N	E	W	G	R	S	G	K	D	P	N	H	Y	R	P	E	G	L	P	D	K	Y
Feline SAA (UniProtKB Q9XSG7) I29/K29	N	S	G	H	G	A	E	D	S	K	A	D	Q	E	A	N	E	W	G	R	S	G	K	D	P	N	H	Y	R	P	E	G	L	P	D	K	Y
Feline SAA (UniProtKB Q9XSG7) Q45/R45	N	S	G	H	G	A	E	D	S	K	A	D	Q	E	A	N	E	W	G	R	S	G	K	D	P	N	H	Y	R	P	E	G	L	P	D	K	Y
Feline SAA (UniProtKB Q9XSG7) A51/V51	N	S	G	H	G	A	E	D	S	K	A	D	Q	E	A	N	E	W	G	R	S	G	K	D	P	N	H	Y	R	P	E	G	L	P	D	K	Y
Feline SAA (UniProtKB Q9XSG7) N75/S75	S	S	G	H	G	A	E	D	S	K	A	D	Q	E	A	N	E	W	G	R	S	G	K	D	P	N	H	Y	R	P	E	G	L	P	D	K	Y
Feline SAA (UniProtKB P19707)	N	S	G	H	G	A	E	D	S	K	A	D	Q	A	A	N	E	W	G	R	S	G	K	D	P	N	H	F	R	P	A	G	L	P	S	K	Y

Figure 4.

Sequence alignment of recombinant feline SAA proteins used for cross-reactivity studies with our MAbs. Identical a.a.r. are highlighted in blue.

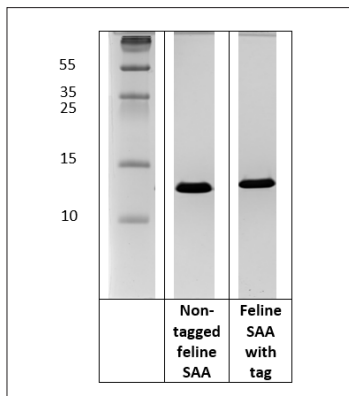


Figure 5.

Tricine-SDS-PAGE of recombinant feline SAA proteins (with and without tag) in reducing conditions. 6 µg of protein was loaded per lane. Gels were stained using Coomassie Brilliant Blue R-250.

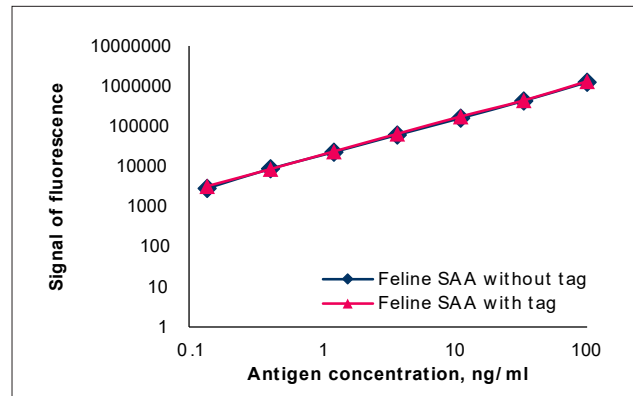


Figure 6.

Calibration curves for the feline SAA prototype assay using pair of antibodies F240-F501 and feline SAA with and without tag as the antigens.

## EQUINE AND CANINE SAA

### Development of a sandwich immunoassays for canine and equine SAA

A high level of sequence homology between dog and horse SAA proteins allows the use of the same antibody combinations for the detection of SAA in both species.

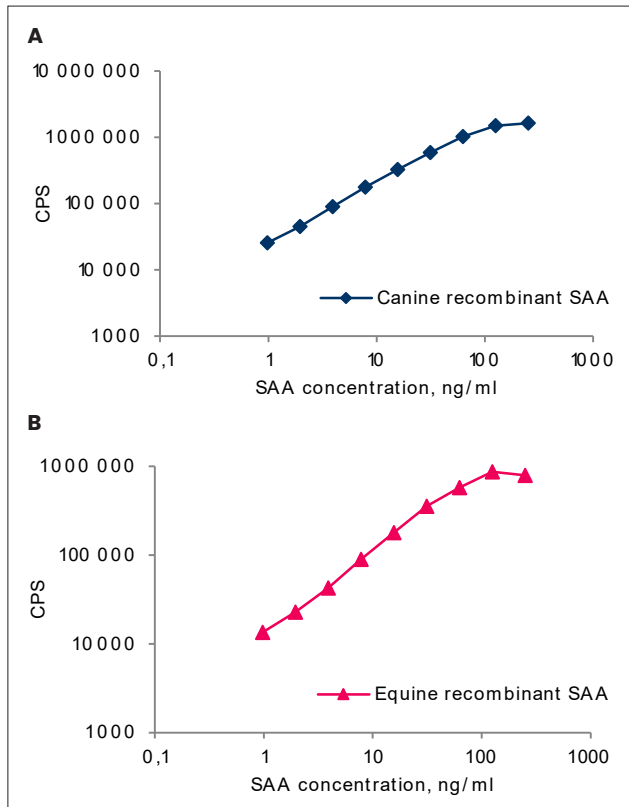
For the development of a sandwich immunoassay for the measurement of SAA in blood samples, we recommend several

**Table 5.**  
Antibody pair recommendations for quantitative equine and canine SAA sandwich immunoassays.

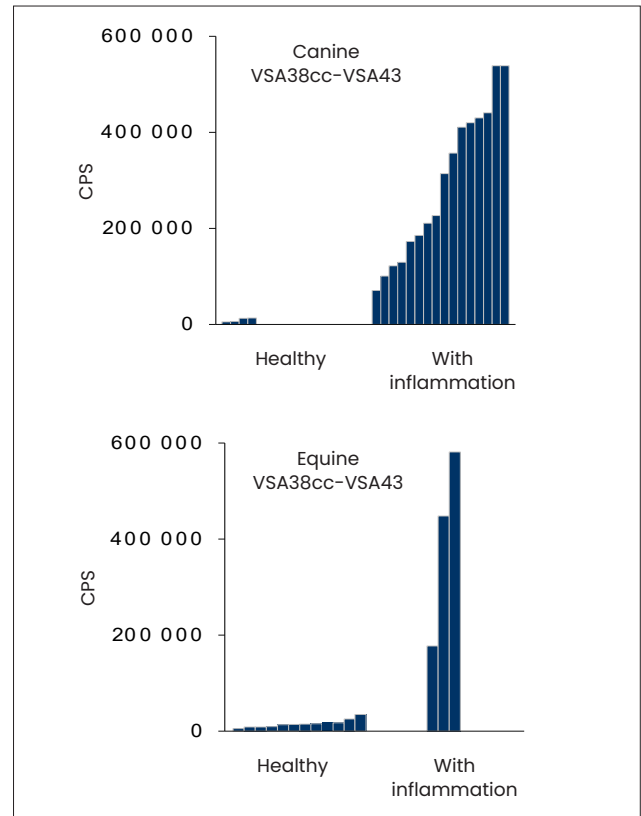
Capture MAb	Detection MAb	Recommended incubation temperature
SAA19cc	VSA34cc	RT
VSA2	VSA38cc	37°C
VSA2	VSA31cc	37°C
VSA38cc	VSA43	37°C

MAb combinations (Table 5). Dilution curves of recombinant canine (A) and equine (B) SAA for the MAb combination VSA2-VSA38cc are provided in Figure 7.

Figure 8 illustrates the detection of SAA in serum samples obtained from dogs (A) and horses (B) using the MAb combination VSA38cc-VSA43. SAA immunoreactivity in serum samples obtained from diseased animals was considerably higher when compared to SAA immunoreactivity in normal serum samples.



**Figure 7.**  
Dilution curves of recombinant canine (A) and equine (B) SAA for the MAb combination VSA2-VSA38cc. Plate wells coated with VSA2 were blocked with a buffer containing 1% casein and 0.05% Tween 20 at 37°C for one hour. The recombinant canine SAA (Cat.# 8CS4) and equine SAA (Cat.# 8ES6) were serially diluted in the same buffer and incubated in the coated plate wells at 37°C for one hour. MAb VSA38cc labelled with europium chelate was used as a detection antibody.



**Figure 8.**  
Comparison of SAA immunoreactivity in serum samples obtained from healthy and diseased dogs (A) and horses (B) detected by using the MAb combination VSA38cc-VSA43. Protocol was as described in the caption of Figure 7. Serum samples from healthy animals were diluted 50-fold with a blocking buffer that contained 1% casein and 0.05% Tween 20. Samples from diseased animals were either diluted 2000-fold (dog serum) or 1000-fold (horse serum).





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## ORDERING INFORMATION

### MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Serum amyloid A (SAA), animal	4VS4	F501	IgG1	Recombinant chimeric antibody
		F529	IgG1	Recombinant chimeric antibody
		F550	IgG1	Recombinant chimeric antibody
		F571	IgG1	Recombinant chimeric antibody
		F173	IgG2a	<i>In vitro</i>
		F227	IgG1	<i>In vitro</i>
		F231	IgG1	<i>In vitro</i>
		F240	IgG2a	<i>In vitro</i>
		SAA19cc	IgG2a	<i>In vitro</i>
		SAA21cc	IgG2b	<i>In vitro</i>
		VSA31cc	IgG2a	<i>In vitro</i>
		VSA34cc	IgG2b	<i>In vitro</i>
		VSA38cc	IgG2a	<i>In vitro</i>
		VSA2	IgG1	
VSA43	IgG2b			

### ANTIGENS

Product name	Cat. #	Purity	Source
Serum amyloid A (SAA), feline, recombinant	8FS5	>95%	Recombinant
Serum amyloid A (SAA), feline, recombinant, non-tagged	8FT7	>95%	Recombinant
Serum amyloid A (SAA), canine, recombinant	8CS4	>95%	Recombinant
Serum amyloid A (SAA), equine, recombinant	8ES6	>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.