Advanced ImmunoChemical Inc.
Providing for the Advancement of Medical Science

ASSAY NOTES
Product Information

Courtesy of HyTest, Ltd

Feline, canine and equine serum amyloid A (SAA) is a marker of inflammation

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

At Advanced Immunochemical, we offer several murine monoclonal antibodies for the development of immunoassays for detection of feline, canine and equine SAA. These antibodies were tested in sandwich immunoassays, direct ELISA and Western blotting, and they recognize SAA from animal serum samples. We also offer recombinant feline, canine and equine SAA proteins.

Monoclonal antibodies specific to SAA

We offer eight well-characterized anti-SAA MAbs for the detection of feline, canine and equine SAA. These MAbs were selected from a pool of over forty MAbs that were raised against human SAA (SAA19, SAA21, SAA11), canine SAA (VSA34, VSA31, VSA38), or synthetic peptides derived from the region 79-104 a.a.r. of canine SAA (VSA2, VSA43). Isotypes and specificities of the antibodies are shown in Table 1. Six MAbs recognize feline SAA in direct ELISA and Western Blotting. Meanwhile, all MAbs recognize canine and equine SAA in direct ELISA and Western Blotting.

MAb	Isotype	Specificity		
		Canine SAA	Feline SAA	Equine SAA
VSA2	IgG1	+++	-	+++
SAA11	IgG2b	+++	++	+++
SAA19	IgG2a	+++	+++	+++
SAA21	IgG2b	+++	+++	+++
VSA31	IgG2a	+++	+++	+++
VSA34	IgG2b	+++	+++	+++
VSA38	IgG2a	+++	+++	+++
VSA43	IgG2b	+++	+/-	+++

Table 1. Characteristics and specificities of anti-SAA MAbs.

Development of a sandwich immunoassay for feline SAA

For sandwich immunoassay development for the measurement of SAA in cat serum samples, the following MAb combinations are recommended: SAA19–VSA34 and SAA21–VSA34 (capture-detection). A dilution curve of recombinant feline SAA for the MAb combination SAA19–VSA34 is provided in Figure 1.

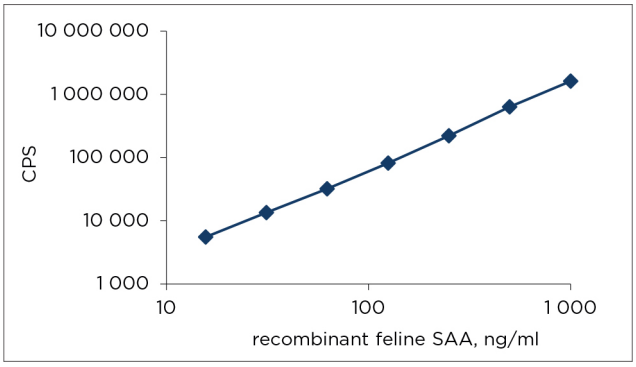

Figure 1. Dilution curve of recombinant feline SAA for the MAb combination SAA19–VSA34. *Plate wells coated with SAA19 were blocked with 2.5% sodium caseinate at 37°C for one hour. Recombinant feline SAA (Cat #8-SAA-f) was serially diluted in Tris-buffer that contained 0.01% CHAPS and 1:1000 normal serum and was incubated in the coated plate wells at room temperature for one hour. The detection antibody VSA34, which was labelled with europium chelate, was diluted in Tris-buffer that contained 0.01% CHAPS. The same buffer containing 0.01% CHAPS was used for plate washing.*

Figure 2 illustrates the detection of SAA in serum samples that were obtained from cats using the MAb combination SAA19–VSA34. SAA immunoreactivity in serum samples obtained from animals with inflammatory diseases was considerably higher as compared to SAA immunoreactivity in normal serum samples.

It was observed that the non-ionic surfactant Tween 20, which is a regular component of antigen dilution and washing buffers, suppressed the signal in these two MAb combinations. Therefore, for the MAb combinations SAA19–VSA34 and SAA21–VSA34, Tween 20 was substituted with the zwitterionic surfactant CHAPS (0.01%) in antigen dilution buffer and washing buffer.

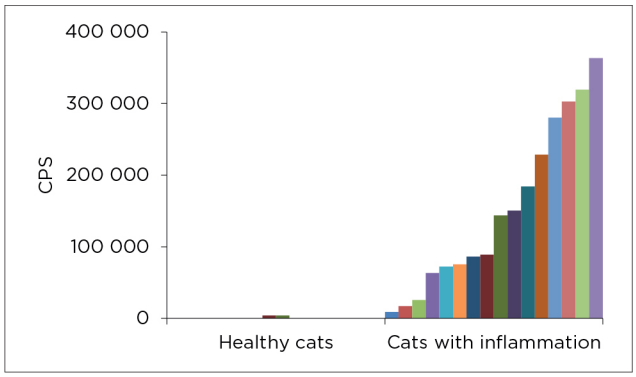

Figure 2. Comparison of SAA immunoreactivity in serum samples obtained from healthy and diseased cats detected by using the MAb combination SAA19–VSA34. *Protocol was as described in the caption of Figure 1. Serum samples were diluted 800-fold in Tris-buffer containing 0.01% CHAPS.*

Development of a sandwich immunoassay 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. All of our antibodies detect both dog and horse SAA. The following 3 antibody combinations show high sensitivity and specificity in sandwich immunoassay: VSA2–VSA38, VSA2–VSA31 and VSA38–VSA43. Dilution curves of recombinant canine (A) and equine (B) SAA for the MAb combination VSA2–VSA38 are shown in Figure 3.

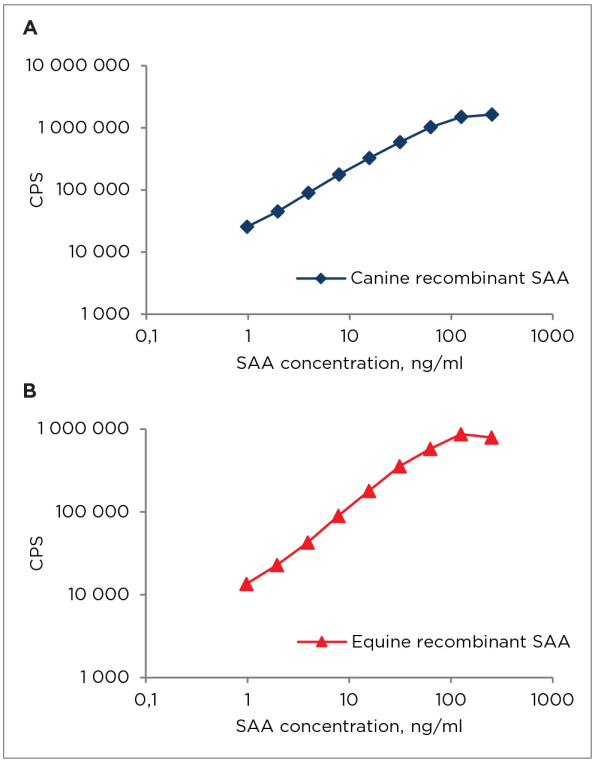

Figure 3. Dilution curves of recombinant canine (A) and equine (B) SAA for the MAb combination VSA2–VSA38. *Plate wells coated with VSA2 were blocked with a buffer containing 1% casein and 0.05% Tween 20 at 37°C for one hour. The recombinant canine SAA (Cat.# 8-SAA-c) and equine SAA (Cat.# 8-SAA-e) were serially diluted in the same buffer and incubated in the coated plate wells at 37°C for one hour. MAb VSA38 labelled with europium chelate was used as a detection antibody.*

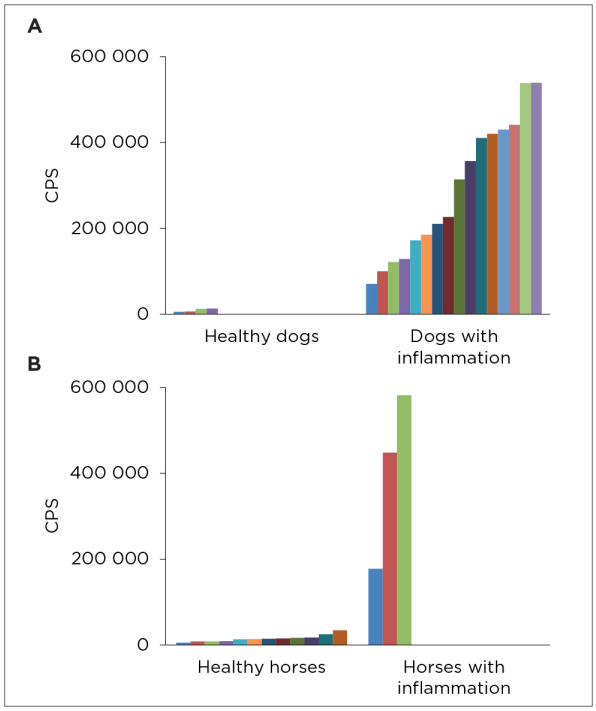

Figure 4. Comparison of SAA immunoreactivity in serum samples obtained from healthy and diseased dogs (A) and horses (B) detected by using the MAb combination VSA38–VSA43. *Protocol was as described in the caption of Figure 3. Serum samples from healthy animals were diluted 50-fold with a blocking buffer 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).*

Figure 4 illustrates the detection of SAA in serum samples obtained from dogs (A) and horses (B) using the MAb combination VSA38–VSA43. SAA immunoreactivity in serum samples obtained from diseased animals was considerably higher when compared to SAA immunoreactivity in normal serum samples.

Avoiding adsorption of SAA onto microtiter plates

It is known from earlier publications that human SAA adsorbs non-specifically onto polystyrene surfaces of microtiter plates (1, 2). SAA from other species (dogs, cats and horses) behaves similarly to human SAA and preferentially adsorbs from serum onto the plate's surface. Therefore, when developing a SAA immunoassay in microtiter plates, it is important to prevent non-specific binding of SAA to the wells of a plate. A plate blocking procedure and antigen dilution buffer should be optimized to ensure that SAA non-specific binding to the plate wells is suppressed.

Casein appears to be an effective blocking agent for SAA immunoassays. For the MAb combinations SAA19–VSA34 and SAA21– VSA34, either the Tris buffer containing 1% casein or 2.5% sodium caseinate was used for microliter plate blocking.

For the MAb combinations VSA2–VSA38, VSA2–VSA31 and VSA38–VSA43, Tris buffer containing 1% casein and 0.05-0.1% Tween 20 was the most efficient solution for both microtiter plate blocking and antigen dilution.

A suggested solid phase blocking procedure and assay buffer composition were optimized for in-house immunoassays. Therefore, other conditions could demonstrate an improved performance in the immunoassays of our customers than those described here.

Development of a single immunoassay for feline, canine and equine SAA

For the development of a universal immunoassay that could detect SAA from cats, dogs and horses, antibody combinations SAA19–VSA34 and SAA21–VSA34 are recommended for feline SAA. Figure 5 illustrates the detection of SAA in serum samples that were obtained from dogs (A) and horses (B) using the MAb combination SAA19– VSA34. SAA immunoreactivity in serum samples obtained from diseased animals was considerably higher as compared to SAA immunoreactivity in normal serum samples. Results of feline serum samples testing using the same MAb combination are shown in Figure 2.

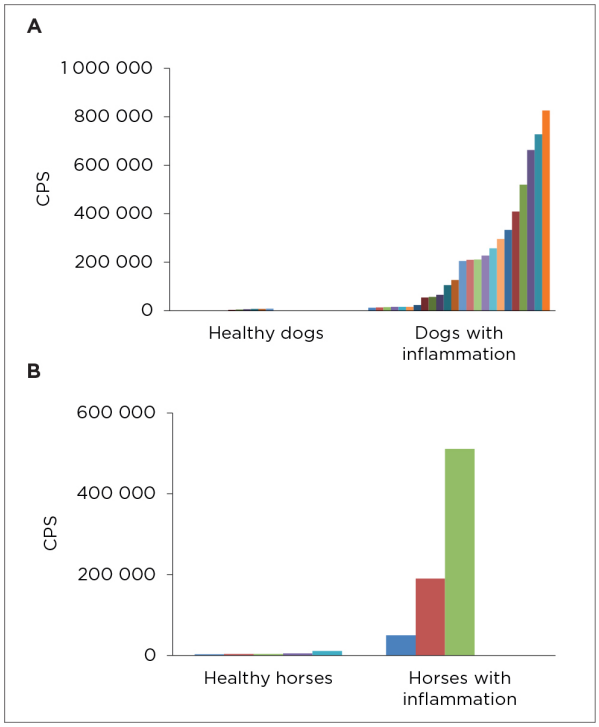

Figure 5. Comparison of SAA immunoreactivity in serum samples obtained from healthy and diseased dogs (A) and horses (B) detected by using the MAb combination SAA19–VSA34. *Protocol was as described in the caption of Figure 1. Dog and horse serum samples were diluted 500-fold and 800-fold respectively in Tris-buffer containing 0.01% CHAPS.*

Table 2 shows the antibody pair recommendations for sandwich immunoassays.

Capture	Detection
Assay for dogs and horses	
VSA2	VSA38
VSA2	VSA31
VSA38	VSA43
Assay for cats, dogs and horses	
SAA19	VSA34
SAA21	VSA34

Table 2. The most sensitive capture-detection pairs. *Data is based on the results obtained using time-resolved fluorescence immunoassay.*

Direct ELISA

All MAbs recognize canine, feline (with the exception of MAbs VSA2 and VSA43) and equine SAA proteins coated onto the wells of polystyrene 96-well plates. The titration curves of four MAbs obtained with the recombinant canine SAA are provided in Figure 6.

1. Marhaug, G. Three assays for the characterization and quantitation of human serum amyloid A. Scand J Immunol, 1983, 18:329–338.
2. Časl, MT and Grubb, A. A rapid enzyme-linked immunosorbent assay for serum amyloid A using sequence-specific antibodies. Ann Clin Biochem, 1993, 30: 278-286.

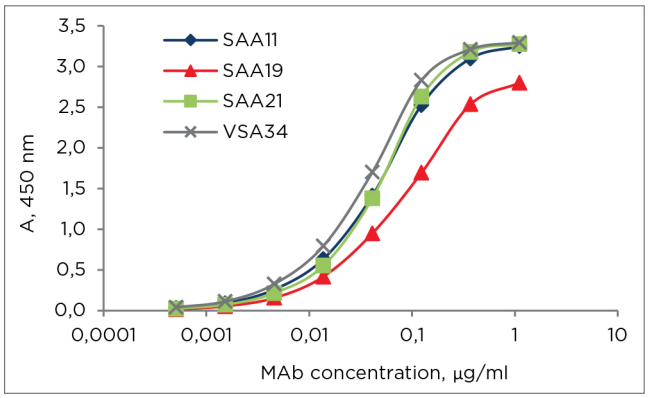

Figure 6. Titration curves of the MAbs obtained with recombinant canine SAA. *A microtiter plate was coated with 0.05 µg of recombinant canine SAA in PBS. MAbs were serially diluted in PBS, pH 7.4, containing 0.1% Tween 20. HRP-conjugated anti-mouse IgG (Fc specific) was used as a secondary antibody. o-Phenylenediamine was used as a substrate.*

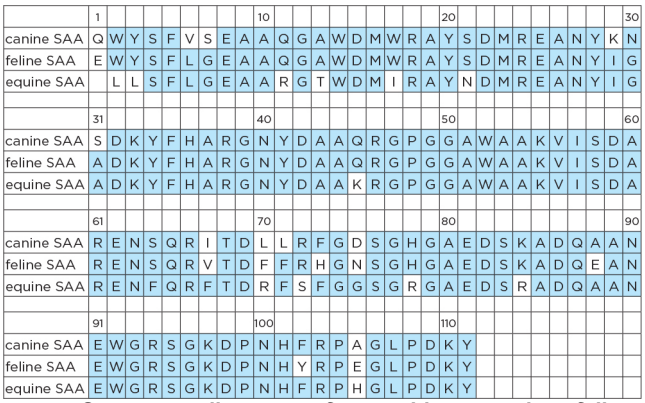

Figure 7. Sequence alignment of recombinant canine, feline and equine SAA. *Identical a.a.r. are highlighted in blue. Recombinant proteins contain an additional 10 a.a.r. on the N-terminus.*

Recombinant canine, feline and equine SAA

Advanced ImmunoChemical offers recombinant canine (121 a.a.r.), feline (121 a.a.r.) and equine (120 a.a.r.) SAA proteins expressed in E. coli. Recombinant SAA proteins contain an additional affinity tag that consists of 10 a.a.r. on the N-terminus of the SAA molecule. Amino acid sequences of recombinant SAA proteins (without tag) are provided in Figure 7.

Recombinant SAAs are purified to homogeneity using several chromatographic steps. The purity of recombinant SAAs is assessed by densitometry following SDS-electrophoresis in reducing conditions and exceeds 95%. Figure 8 shows the SDS-PAGE of purified canine, feline and equine SAA.

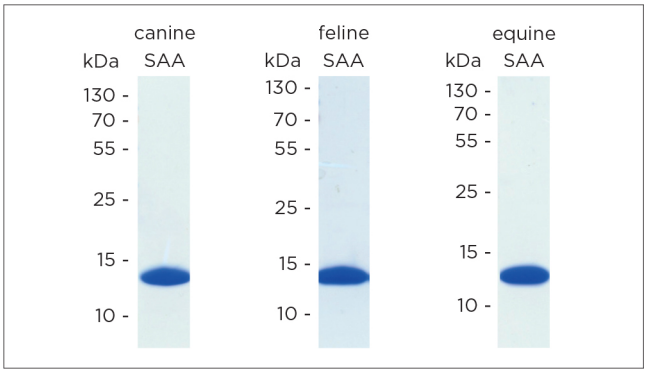

Figure 8. Tricine-SDS-PAGE of recombinant canine, feline and equine SAA in reducing conditions. *10 µg of protein was loaded per lane. Gels were stained using Coomassie Brilliant Blue R-250.*

Ordering Information:

MONOCLONAL ANTIBODIES

Product Name	Cat #	MAb	Subclass	Remarks
Serum amyloid A (SAA), animal	2-SAA-a	VSA2	IgG1	EIA, WB, Specific to canine and equine SAA.
		SAA11	IgG2b	EIA, WB, Specific to canine, feline and equine SAA.
		SAA19	IgG2a	EIA, WB, Specific to canine, feline and equine SAA.
		SAA21	IgG2b	EIA, WB, Specific to canine, feline and equine SAA.
		VSA31	IgG2a	EIA, WB, Specific to canine, feline and equine SAA.
		VSA34	IgG2b	EIA, WB, Specific to canine, feline and equine SAA.
		VSA38	IgG2a	EIA, WB, Specific to canine, feline and equine SAA.
		VSA43	IgG2b	EIA, WB, Specific to canine and equine SAA.

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Product Name	Cat. #	Purity	Source
Canine serum amyloid A (SAA)	8-SAA-c	>95%	Recombinant
Feline serum amyloid A (SAA)	8-SAA-f	>95%	Recombinant
Equine serum amyloid A (SAA)	8-SAA-e	>95%	Recombinant

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