TechNotes

HyTest

Clinical and Research Area | Veterinary | Inflammation

Canine CRP (cCRP) is a marker for systemic inflammation

C-reactive protein (CRP) is a major acute phase protein in dogs. Its concentration increases rapidly and significantly during systemic inflammation and subsequently decreases quickly following the elimination of the source of inflammation. Kinetics of canine CRP (cCRP) in an acute phase response are similar to those of human CRP. The synthesis of CRP in hepatocytes is induced by inflammatory cytokines shortly after the inflammatory stimulus. However, whilst CRP plays an important role in innate immune response, its precise function appears to be complex and still somewhat unclear.

cCRP as a diagnostic marker

Several studies support the view that cCRP is a valuable diagnostic marker for the detection of the acute phase response. Its concentration has been shown to increase rapidly in various disorders including viral and bacterial infections, sepsis and pyometra, as well as in surgical trauma (1-3 and references therein). It has been suggested to be useful in monitoring a possible post-surgical infection (4,5). Further, cCRP has been indicated as a marker of antibiotic treatment response in dogs with bacterial pneumonia (6). If cCRP measurements could be used for guiding the duration of treatment, this would help to reduce the use of antibiotics in dogs.

Biochemical properties of CRP

CRP belongs to a family of pentraxins. These evolutionally conserved proteins are pentamers and have calcium-dependent ligand binding properties. The major function of pentraxins is to protect organisms against foreign and altered antigens (7).

CRP is composed of five identical subunits that form a ring-like structure. The molecular mass of CRP is approximately 115 kDa with each subunit being 23 kDa and consisting of 204 amino acids. The major difference between human and canine CRP is that two out of the five cCRP subunits are glycosylated while human CRP has no glycans (8).

Reagents for developing a reliable CRP assay

In many cases, immunoassays for the detection of cCRP are based on antibodies that are specific to human CRP. However, it should be noted that the two proteins are modified differently in blood. cCRP is glycosylated while human CRP in general is not. Therefore, the level of cross-reactivity of the anti-human CRP antibodies with cCRP may vary depending on the epitope specificity and especially with polyclonal antibodies batch-to-batch differences can be significant.

HyTest offers several monoclonal antibodies (MAbs) and a recombinant antigen that enable the development of a sensitive and specific cCRP immunoassay. Our anti-canine CRP MAbs recognize CRP in dog serum with high specificity and sensitivity. The antibodies are not sensitive to the presence of chelating agents such as EDTA. We have tested these antibodies in sandwich immunoassays, direct and indirect ELISA, and Western blotting.

The recombinant protein can be used as a calibrator in canine CRP immunoassays.

In addition, we also offer polyclonal antibodies specific to canine CRP.

Monoclonal antibodies specific to cCRP

We provide four well-characterized, specific and sensitive anti-canine CRP MAbs. They were selected from among twenty MAbs that have been raised against cCRP. All of our MAbs may be used in different immunoassays for the qualitative and quantitative detection of cCRP. No cross-reactivity was observed either to other dog serum components or to human CRP. In addition, no signal was detected from cat, horse, rabbit and goat serums with the antibodies.

Three MAbs are suitable for sandwich type immunoassays while one works well in direct ELISA and Western blotting. Table 1 summarizes the basic characteristics and application recommendations for our anti-canine CRP MAbs.

Table 1. Characteristics and recommendations for utilizing anti-canine CRP MAbs in different applications.

MAb	Isotype	Epitope	Sandwich immunoassay	Direct ELISA	WB
cCRP1cc	IgG1	Conf.	+++	+	-
cCRP3	IgG2b	Linear	-	+++	+++
cCRP11cc	IgG1	Conf.	+++	-	-
cCRP34cc	IgG1	Conf.	+++	-	-

Conf. = Conformational

A sandwich immunoassay for cCRP detection

We tested the MAbs for their applicability in sandwich type immunoassays. With the exception of cCRP3, all of the MAbs can be used as capture and detection antibodies in these assays. Figure 1 shows a calibration curve for native cCRP using MAbs cCRP11 and cCRP1 for capture and detection respectively. The recommended MAb combinations show high sensitivity (up to 0.1 ng/ml) and a long linearity range in a sandwich fluoroimmunoassay.

Recommended capture-detection antibody pairs are listed in Table 2.

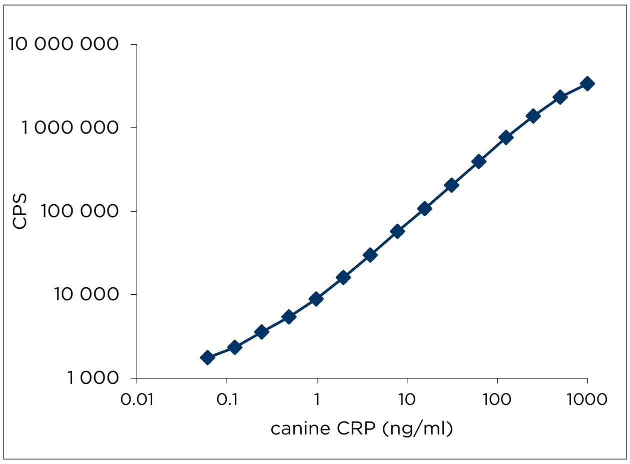

Figure 1. Calibration curve of native cCRP in a sandwich fluoroimmunoassay. cCRP11 and Eu^{3+}-labeled cCRP1 were used as capture and detection antibodies respectively.

Table 2. The most sensitive capture-detection pairs. Data is based on the results obtained using our in-house DELFIA® immunoassay platform.

Capture	Detection
Hetero-sandwich assay	
cCRP11cc	cCRP1cc
cCRP34cc	cCRP1cc
Homo-sandwich assay	
cCRP11cc	cCRP11cc

Detection of cCRP in serum samples

MAbs were tested for their ability to detect endogenous cCRP in serum samples. According to our test, most MAb combinations were able to detect endogenous cCRP in a reliable manner. Figure 2 shows an example of measuring cCRP concentrations from 34 serum samples from dogs with systemic inflammation of different origins and 8 serum samples from healthy dogs. cCRP34 and cCRP1 were used as capture and detection antibodies respectively. The results demonstrate that the concentration of cCRP in the group of animals with inflammation is considerably higher compared to healthy dogs.

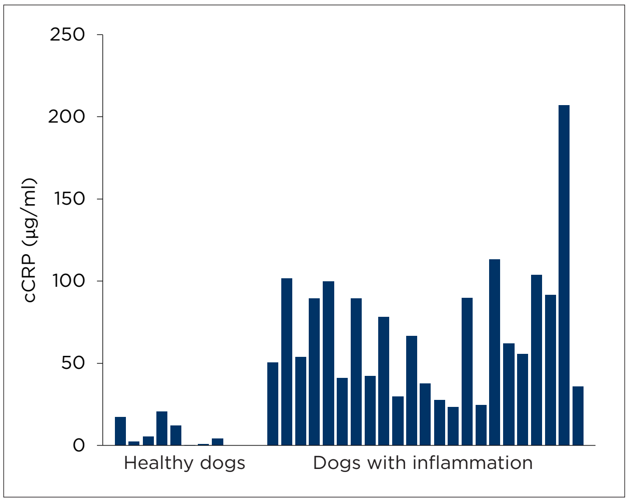

Figure 2. cCRP levels in serum of healthy dogs or dogs with systemic inflammation. cCRP34 and Eu^{3+}-labeled cCRP 1 were used as capture and detection antibodies respectively.

Detection of cCRP in the absence of Ca^{2+}

Several cCRP assays described in literature utilize phosphocholie (PC) conjugated to a carrier protein as a capture molecule. PC is a specific ligand to which cCRP binds with high affinity. However, this binding is very much dependent on Ca^{2+} and is abolished if Ca^{2+} is not available. Conversely, the performance of our MAbs is not affected by the presence or absence of Ca^{2+}. Figure 3 shows that using MAb cCRP11 as a capture molecule enables the sensitive detection of cCRP independent of Ca^{2+} as compared to PC, which is unable to capture cCRP in the absence of Ca^{2+}.

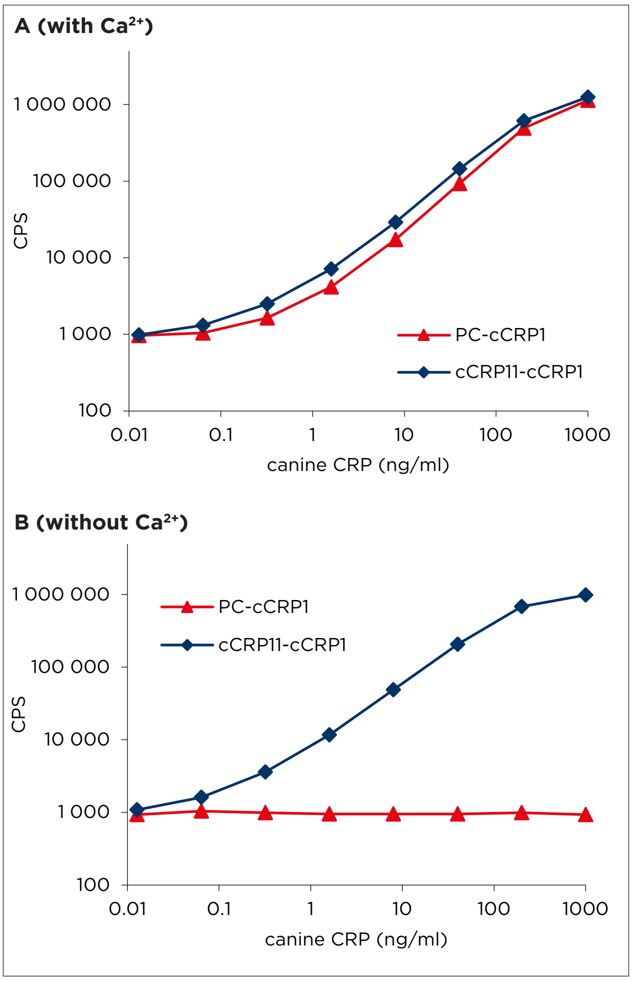

Figure 3. Comparison of PC and MAb cCRP11 as capture molecules in the presence (A) or absence (B) of Ca^{2+}. PC was conjugated to BSA. Native cCRP was used as the antigen and Eu^{3+} labelled cCRP1 as the detection antibody. The assay buffer was supplemented with 2 mM Ca^{2+} (A) or 5 mM EDTA (B).

Phosphocholine binding does not influence cCRP detection by MAbs

During a systemic inflammation or polytrauma, PC originating from invading bacteria or damaged cells may appear in the blood. As a result, some CRP molecules circulating in blood are likely to be associated with PC. This may affect the detection of cCRP if PC is used as a capture molecule in the assay.

Our antibodies are not sensitive to PC-CRP association. No changes in the signals were detected in the presence or absence of PC (Figure 4) in a sandwich immunoassay utilizing two monoclonal antibodies.

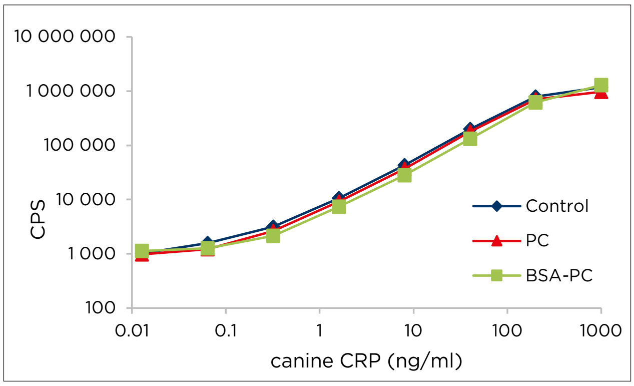

Figure 4. PC in the sample does not affect the detection of cCRP with monoclonal antibodies in a sandwich immunoassay. Native cCRP was used as the antigen and Eu^{3+} labelled cCRP1 as the detection MAb. The assay buffer was supplemented with PC or PC conjugated to BSA.

Direct ELISA and Western blotting

One MAb, cCRP3, is suitable for CRP detection in direct ELISA (Figure 5) and Western blotting (not shown). It can also be used in indirect ELISA but not in sandwich type immunoassays. It would appear that cCRP3 recognizes a linear epitope of the cCRP molecule that becomes available for antibody binding after immobilization of the molecule on a microtiter plate surface and following SDS-PAGE in reducing conditions.

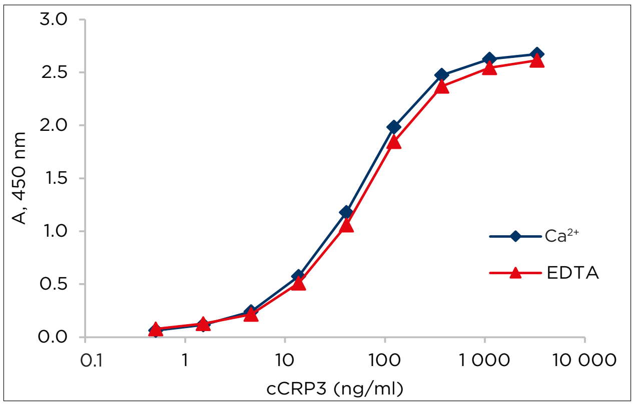

Figure 5. Detection of native cCRP in direct ELISA with MAb cCRP3 in the presence of Ca^{2+} or EDTA. 50 ng of native cCRP was coated onto 96 well microtiter plate wells and titrated with MAb cCRP3 in Tris buffer in the presence of 2 mM Ca^{2+} or 5 mM EDTA.

Polyclonal antibodies detecting canine CRP

In addition to monoclonal antibodies, we also provide polyconal antibodies that can be used for the detection of cCRP in immunoassays.

Calibration curves for the recombinant cCRP using a pair of monoclonal antibodies (cCRP11-cCRP1) and a pair in which the polyclonal antibodies are used as capture antibodies (pAbs-cCRP1) is shown in Figure 6. Both pairs show similar performance in sandwich immunoassay.

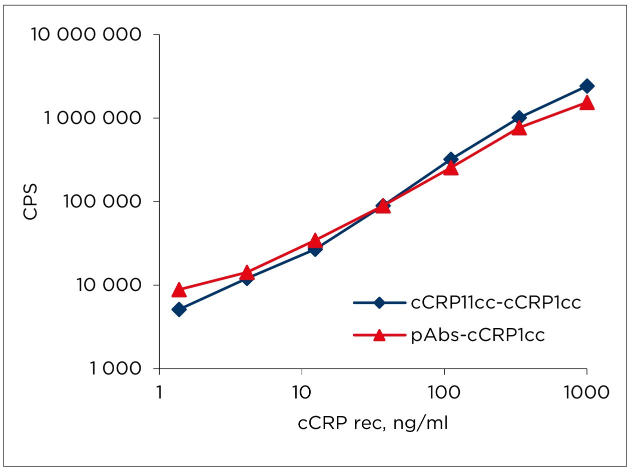

Figure 6. Calibration curves for recombinant cCRP with a MAb-MAb pair (blue) and pAbs-MAb pair (red). Detection antibody cCRP1 was labelled with Eu^{3+}.

We also confirmed that the polyclonal antibodies can be used for the detection of native cCRP in dog serum.

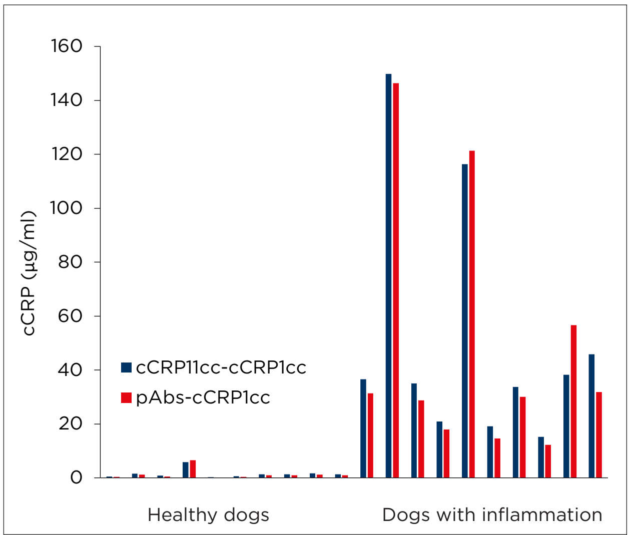

Figure 7. cCRP concentration measured in serum of healthy dogs or dogs with systemic inflammation with two immunoassays. Detection antibody cCRP1 was labelled with Eu^{3+}.

Recombinant canine CRP

HyTest provides a recombinant cCRP (204 a.a.r.) that is produced in a eukaryotic expression system and purified to a level exceeding 95% purity. Our recombinant cCRP is partially glycosylated and its biochemical and immunochemical properties are similar to those of native cCRP. The recombinant cCRP can be used as a calibrator or standard in cCRP immunoassays.

Recombinant cCRP is partially glycosylated

It has been shown that canine CRP is a glycoprotein and it has been predicted that two of the five subunits are glycosylated. In SDS-PAGE the native cCRP migrates as a duplet band with the upper band representing the glycosylated subunits (8).

Our recombinant cCRP is produced in a system that allows for the glycosylation of the protein. When the purified protein was run in SDS-PAGE under reducing conditions it migrated as two separate bands in a fairly similar way to the native cCRP (Figure 8). The lower bands showed identical motility and represent the non-glycosylated subunits. Meanwhile, the upper bands that represent the glycosylated subunits migrated differently. This indicates that as is the case with the native cCRP, only some of the subunits of the recombinant protein are glycosylated. However, the glycosylation patterns between the two proteins appear to be different.

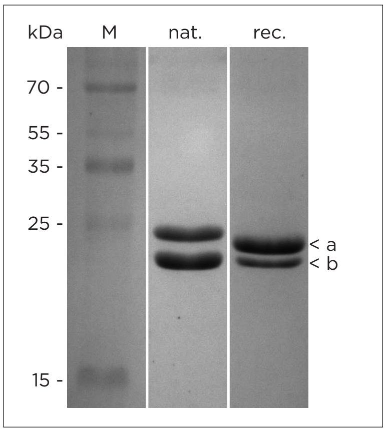

Figure 8. Comparison of recombinant and native cCRP in SDS-PAGE. 3 µg of purified proteins were run in 12.5% gel under reducing conditions. The gel was stained with Coomassie brilliant blue R-250.
a: glycosylated CRP
b: non-glycosylated CRP

The glycosylation of the recombinant protein was confirmed by glycoprotein specific staining following a SDS-PAGE run (Figure 9).

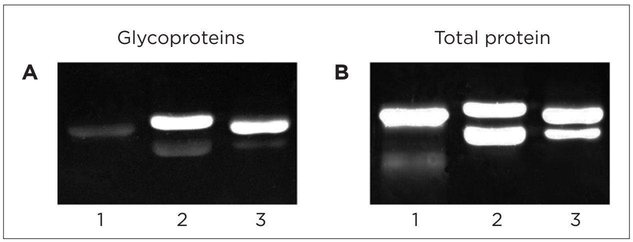

Figure 9. Staining of the carbohydrate moieties of the native and recombinant cCRP. 3 µg of native cCRP (lane 2) and recombinant cCRP (lane 3) were run in a 12.5% SDS-PAGE under reducing conditions. The gel was first stained with Pro-Q emerald 300 to reveal glycoproteins (A) and then with SYPRO® Ruby for the visualization of total proteins (B). 3 µg of human CRP (Cat.# 8C72) was included as a reference (lane 1).

Recombinant cCRP compared to native cCRP in a sandwich immunoassay

Titration curves of the recombinant and native cCRP were identical when compared in our in-house DELFIA immunoassay (Figure 10). We used cCRP11 and cCRP1 in this assay as capture and detection antibodies respectively.

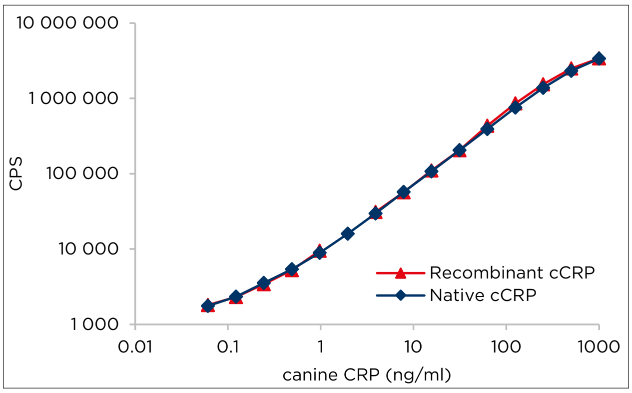

Figure 10. Calibration curves of the native and recombinant cCRP in a sandwich fluoroimmunoassay. cCRP11 and Eu^{3+}-labeled cCRP1 were used as capture and detection antibodies respectively.

Recombinant cCRP binds to PC

One of the functions of CRP is its Ca^{2+} -dependent binding to C-polysaccharides of the bacterial cell wall. The major reactive group for CRP binding is PC that is presented on C-polysaccharide residues.

To determine whether the recombinant cCRP is able to bind PC like the native cCRP we titrated both proteins in a sandwich immunoassay using PC conjugated to BSA as the capture molecule. No differences were observed between the recombinant and native cCRP in this binding activity (Figure 11).

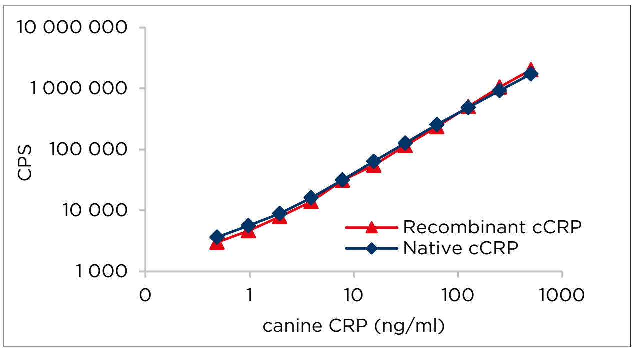

Figure 11. Titration curves for the recombinant and native cCRP in a sandwich fluoroimmunoassay using PC as the capture molecule. PC was conjugated to BSA in this assay. Eu^{3+}-labeled cCRP1 was used as the detection antibody.

Recombinant cCRP is a pentamer

To investigate whether our recombinant cCRP was able to form pentamers, we analyzed it in a homo-sandwich immunoassay. It is possible to use one single MAb for both capturing and detection if there are more than one antibody specific epitopes available for binding in the molecule. Figure 12 shows a calibration curve of the recombinant and native cCRP using cCRP11 MAb as both the capture and detection antibody. The result indicates that the recombinant protein is an oligomer. Based on this and other assays that we have made (HPLC, gel filtration and analysis in PAGE with a reduced amount of SDS; not shown) we can conclude that the recombinant cCRP is a pentamer.

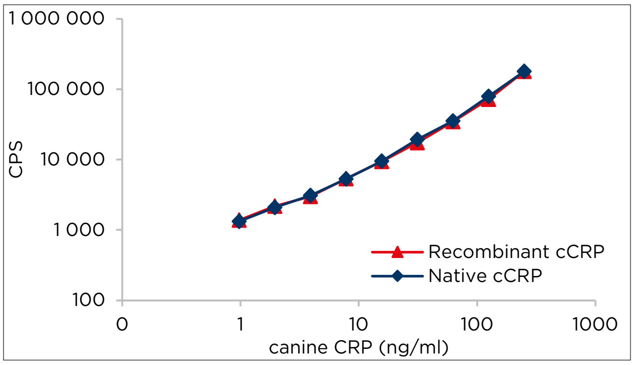

Figure 12. Calibration curves of the recombinant and native cCRP in a homo-sandwich fluoroimmunoassay. MAb cCRP11 was used as a capture and detection antibody. The antibody was labeled with Eu^{3+} for detection.

References

1. Cerón, J. J., Eckersall, P. D. & Martýnez-Subiela, S. Acute phase proteins in dogs and cats: current knowledge and future perspectives. Vet. Clin. Pathol. 34, 85–99 (2005).
2. Eckersall, P. D. & Bell, R. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. Vet. J. 185, 23–27 (2010).
3. Kjelgaard-Hansen, M. Canine C-reactive protein – a study on the applicability of canine serum C-reactive protein. (Copenhagen: Department of Small Animal Clinical Sciences, The Royal Veterinary and Agricultural University, 2004).
4. Löfqvist, K., Kjelgaard-Hansen, M. & Nielsen, M. B. M. Usefulness of C-reactive protein and serum amyloid A in early detection of postoperative infectious complications to tibial plateau leveling osteotomy in dogs. Acta Vet. Scand. 60, 30 (2018).
5. Christensen, M. B., Eriksen, T. & Kjelgaard-Hansen, M. C-reactive protein: quantitative marker of surgical trauma and post-surgical complications in dogs: a systematic review. Acta Vet. Scand. 57, 71 (2015).
6. Viitanen, S. J., Lappalainen, A. K., Christensen, M. B., Sankari, S. & Rajamäki, M. M. The Utility of Acute-Phase Proteins in the Assessment of Treatment Response in Dogs With Bacterial Pneumonia. J. Vet. Intern. Med. 31, 124–133 (2017).
7. Du Clos, T. W. Pentraxins: Structure, Function, and Role in Inflammation. ISRN Inflamm. 2013, 1–22 (2013).
8. Caspi, D. et al. Isolation and characterization of C-reactive protein from the dog. Immunology 53, 307–313 (1984).

Ordering information

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Canine C-reactive protein (cCRP)	4CC5	cCRP1cc	IgG1	*In vitro*, EIA
		cCRP3	IgG2b	EIA, WB
		cCRP11cc	IgG1	*In vitro*, EIA
		cCRP34cc	IgG1	*In vitro*, EIA

POLYCLONAL ANTIBODY

Product name	Cat. #	Host Animal	Remarks
Polyclonal anti-canine C-reactive protein (cCRP)	PRP4	Goat	EIA

ANTIGEN

Product name	Cat. #	Purity	Source
Canine C-reactive protein (cCRP)	8CC5	>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.

HyTest Ltd
Intelligate 1, 6th floor, Joukahaisenkatu 6
FI-20520 Turku, FINLAND

Tel. +358 2 512 0900, Fax +358 2 512 0909
E-mail: hytest@hytest.fi
www.hytest.fi