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CANINE C-REACTIVE PROTEIN ASSAY

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Cat. No. 931CRP01D-96

C-REACTIVE PROTEIN

C-reactive protein (CRP) is an acute-phase protein produced by the liver in conditions of inflammation, bacterial infection, and/or tissue trauma. Quantification of CRP is useful in determining inflammatory conditions difficult to diagnose, detect abnormalities in patients with chronic inflammatory conditions, and to monitor patients' response to treatment.

INTENDED USE

The HELICA Canine C-Reactive Protein Assay is a sandwich-based enzyme-linked immunoassay intended for the detection and quantification of canine CRP in canine serum.

ASSAY PRINCIPLE

The HELICA Canine C-Reactive Protein Assay is species specific and provides a quantitative assessment of CRP levels in canine serum or plasma. The HELICA Canine C-Reactive Protein Assay is a sandwich-based enzyme-linked immunoassay in which the microtiter plates are coated with pneumococcal C-polysaccharide. After samples are prepared following the listed specimen collection procedure, they are applied to the antigen coated plate alongside the prepared standards. After incubation, the wells are decanted and washed to remove unreacted serum or plasma proteins, and an enzyme labeled anti-canine CRP antibody (conjugate) is added and reacts with the antigen-antigen complexes. Following another incubation period, the wells are decanted and washed to remove unreacted conjugate. A hydrogen peroxide substrate with TMB as a chromogen is added to start color development. The intensity of the color is directly proportional to the amount of canine CRP in the sample. Therefore, the greater the intensity of the blue color, the higher the canine CRP concentration in the sample. The reaction is interrupted with a stop solution that turns the blue product yellow. The absorbance is read at a wavelength of 450nm on a spectrophotometer or plate reader.

REAGENTS PROVIDED

1 X Pouch	**Antibody Coated Microwell Plate**		96 wells (12 eight well strips) in a microwell holder coated with pneumococcal C-polysaccharide, *Ready-to-Use.*
1 X Vial	**Canine CRP Standard (10X)**	Green Cap	0.25mL of canine serum with elevated CRP concentration at 4µg/mL. Dilute standard stock 10X in Tris-T followed by three 3-fold serial dilutions to prepare standards at the following concentrations: 400.0, 133.3, 44.4, and 14.8ng/mL.
1 X Vial	**Anti-Canine CRP Conjugate (100X)**	Amber Cap	0.13mL of 100X anti-canine CRP-IgG protein conjugated to peroxidase in buffer with preservative. Dilute conjugate in Tris-T to 1X prior to use.
1 X Bottle	**Substrate Reagent**	Blue Cap	12mL stabilized tetramethylbenzidine (TMB), *Ready-to-Use.*
1 X Bottle	**Stop Solution**	Red Cap	12mL Acidic Solution, *Ready-to-Use.*
1 X Pouch	**Washing Buffer**		Tris with 0.05% Tween20, bring to 1L with distilled water and store refrigerated.

MATERIAL REQUIRED BUT NOT SUPPLIED

- Distilled or deionized water
- Wash bottle
- Dilution tubes
- Pipettor with tips: 2µL to 1000µL
- Adhesive cover for microplate
- Microplate reader with 450nm filter

PRECAUTIONS

1. Bring all reagents to room temperature (19º-27ºC) before use.
2. Store reagents at 2º-8ºC, and do not use beyond expiration date(s). Never freeze kit components.
3. Do not return unused reagents back into their original vials or bottles.
4. Do not interchange kit components between different lots of the same assay.
5. Adhere to all time and temperature conditions stated in the procedure.
6. Never pipette reagents or samples by mouth.
7. The Stop Solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
8. The standard serum and conjugate have not been screened for infectious agents. Since no testing can assure the absence of infectious agents, consider all materials, containers, and devices that are exposed to sample, standard, and conjugate to be contaminated with canine serum proteins. Wear protective gloves and safety glasses when using this kit.
9. The coated microwells have been prepared with inactivated antigens; however, they should be considered potentially infectious and handled accordingly.
10. Dispose of all materials, containers, and devices in the appropriate receptacle after use.
11. HRP-labeled conjugate and TMB substrates are photosensitive and are packaged in protective opaque bottles. Store in the dark and return to storage after use.

SPECIMEN COLLECTION AND PREPARATION

Specimens that are able to be used for this assay include: serum, plasma, urine, culture supernatant, tissue extracts, and synovial fluids.

Blood Samples

Blood samples should be collected using approved venipuncture techniques by qualified personnel. Allow sample to clot and separate serum by centrifugation. Transfer serum aseptically to a tightly closing sterile container. Store at 2-8°C. Alternatively, plasma extracted from blood drawn in heparin, EDTA, or ACD-containing tubes is acceptable. If testing is to be delayed longer than 5 days, freezing the sample at -20°C or below is recommended.

Upon specimen collection, dilute the sample 1:500 in Tris-Tween wash buffer (i.e. add 2µL of sample to 1mL of wash buffer). The final dilution for use in calculation is 1:500.

Specimens other than Blood (Serum or Plasma)

Samples other than blood (serum or plasma) should be prepared at higher concentrations. It is recommended to begin diluting the sample 1:2 in Tris-Tween wash buffer (i.e. add 100µL of sample to 200µL of wash buffer) and increasing the dilution factor accordingly. Dilution factor must be accounted for in the final calculation.

ASSAY PROCEDURE

Note: It is recommended that a multi-channel pipettor be utilized to perform the assay if more than 16 samples and standards (2 test strips) are run.

1. Bring all the reagents to room temperature before use. Reconstitute the Tris-Tween packet by washing out the contents with a gentle stream of distilled or deionized water into a 1-Liter container. Q.S. to 1 Liter with distilled or deionized water and store refrigerated when not in use.
2. Dilute 10X standard stock to 1X working concentration in Tris-Tween to prepare 400.0ng/mL standard. Prepare remaining standards by serially diluting standards 3-fold three times to yield 133.3, 44.4, and 14.8ng/mL. Use Tris-Tween wash buffer for 0.0ng/mL standard. Consider the following dilution scheme as a guide:

Standard Concentration (ng/mL)	Tris-Tween Volume	Volume Transferred	Total Volume	Final Volume *After serial dilution
400.0	162µL	18µL	180µL	120µL
133.3	120µL	60µL	180µL	120µL
44.4	120µL	60µL	180µL	120µL
14.8	120µL	60µL	180µL	180µL

3. Using a new pipette tip for each, add 100µL of each standard and prepared sample into the appropriate wells. Samples and standards can be run in duplicate if desired. Incubate at room temperature for 30 minutes.
4. Decant the contents from the microwells into a discard basin. Wash the microwells by filling each with Tris-Tween wash buffer, then decanting the wash buffer into a discard basin. Repeat for a total of 5 washes.
5. Tap the microwells (face down) on a layer of absorbent towels to remove residual wash buffer.
6. Determine the required volume of conjugate (1mL/strip or 120µL/well) to prepare. Dilute stock conjugate (100X) to working concentration (1X) with Tris-Tween wash buffer.
7. Add 100µL of conjugate to each well. Incubate at room temperature for 30 minutes. Cover to avoid direct light.

8. Repeat steps 4 and 5.
9. Add 100µL of Substrate Reagent to each well. Incubate at room temperature for 10 minutes. Cover to avoid direct light.
10. Add 100µL of Stop Solution to each well in the same sequence and at the same pace as the Substrate Reagent was added.
11. Read the optical density (OD) of each microwell with a microtiter plate reader using a 450nm filter. Record the optical density (OD) of each microwell.

INTERPRETATION OF RESULTS

Construct a standard curve using the OD values against the concentration of the standards. Unknowns are measured by interpolation from the standard curve. Final concentration must be multiplied by the dilution factor at which samples were prepared to get the actual concentration (ng/mL). If a sample contains CRP at a greater concentration than the highest standard, it should be diluted appropriately in Tris-Tween and re-tested. The extra dilution step should be taken into account when expressing the final result.

ASSAY CHARACTERISTICS

Limitations

Lipemic sera may interfere with specific antibody reaction.

Limit of Detection

The lower and upper limit of quantitation is 14.8 and 400.0ng/mL, respectively.

Quality Control

It is recommended to routinely run at least two controls, each giving values at the top or bottom regions of the standard curve. An occasional prozone may be encountered in sera with high CRP values. In this situation, due to antigen excess, all the CRP available may not have reacted with the conjugate. Therefore, test at higher dilution (e.g. 1:1000 and 1:2000) to obtain more accurate results.

Cross-Reactivity

Species	# of Runs	% Cross-reactivity
Rat	15	0%

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