



"PHASE"™ EIA Canine CRP Assay Cat. No. TP-803

Intended use

The activation of the body's immune system-mediated defence mechanisms is termed the acute phase response. Activation can occur due to infections, inflammation, tissue injury, neoplastic growth or immunological disorders. This assay is designed to detect the acute phase protein, C-Reactive protein, from the serum of dogs.

Note: This product is for research purposes only.

Introduction

C-Reactive Protein is one of the family of acute phase proteins found in the blood of both humans and animals. Under normal conditions it is found in low levels in the blood but can increase significantly in response to inflammatory conditions, infections and other disease states where tissue necrosis occurs, and therefore provides a highly sensitive indicator for these conditions.

Assay principle

The Tridelta Phase™ range canine CRP kit is a solid phase sandwich Immunoassay. Samples, including calibrators of known CRP content bind to coated microwells. After washing to remove any unbound material the HRP labeled Anti-canine-CRP antibody is added to each well. After again washing to remove any unbound material TMB substrate solution is added. The intensity of the colour produced is proportional to the concentration of CRP present in the original specimen.

Components Supplied

1. Coated microplate 1 x 96 well plate
2. C-Reactive protein calibrator 1 x 1.5ml (Ready to use)
3. Standard/sample diluent buffer 1 x 50ml (20x concentrate)
4. Anti-canine CRP Conjugate 1 x 11ml (Ready to use)
5. Wash concentrate 1 x 50ml (20x concentrate)
6. TMB Substrate 1 x 11ml (Ready to use)
7. Stop solution 1 x 11ml (Ready to use)
8. Information leaflet 1

Additional materials required

1. Serum collection equipment.
2. Microtiter plate reader capable of measurement at 450nm with reference at 630nm if available.
3. Accurate micropipettes and disposable tips to deliver 0-10µl, 20-200µl and 200-1000µl.
4. A repeat or multichannel pipette (50-200µl) for large assays.
5. Deionized or distilled H₂O.
6. Plate washer (automated or manual).
7. Graph paper: Standard or semi-log.
8. Glass or plastic test tubes.
9. Absorbent paper towels
10. 96 well dust plate cover.
11. 37°C incubator.
12. Timer

Storage and Stability

The kit components are stable when stored at 2-8°C until the expiry date indicated on the kit label.

Safety

- Never pipette by mouth.
- Wear disposable latex gloves and eye protection where appropriate.
- The stop solution and TMB substrate contain reagents that may irritate the skin or mucous membranes. Any reagent, which comes into contact with the skin, should be washed off with water immediately.

Sample Preparation

Samples

Specimens should be collected by venipuncture into serum collection tubes. Blood samples may be kept for up to 24 hours before separation of serum. However, it is best to remove serum from the clot as soon as possible after collection. In general, serum may be stored at 2-8°C for up to 24 hours or stored frozen at -20°C for longer periods without loss of CRP. It is important that all refrigerated samples are brought to room temperature and mixed to assure accurate determination of the CRP concentration.

All samples should be diluted 1:500 in sample diluent buffer (1x) prior to assay by addition of 10ul of sample to 5.0 ml diluent buffer (1x).

Do not use grossly haemolysed or lipaemic samples.

Reagent Preparation

Diluent Buffer (1x)

Dilute 1 volume of diluent buffer concentrate (20x) with 19 volumes of distilled water. Store both the diluent buffer concentrate and working diluent buffer (1x) in the refrigerator. Diluted diluent buffer is stable for up to 2 weeks when stored at 4°C.

Wash Buffer (1x)

Dilute 1 volume of wash buffer concentrate (20x) with 19 volumes of distilled water. Store both the wash buffer concentrate and working wash buffer (1x) in the refrigerator. Diluted wash solution is stable for up to 2 weeks when stored at 4°C.

Calibration curve preparation

Label 6 tubes C1 to C6. To prepare the top calibrator, add 250ul of the calibrator supplied with the kit to tube C1 (see table 1). Add 250ul 1x diluent buffer to each of the remaining tubes (C2-C6) as directed in table 1. The top calibrator is serially diluted 4 times (tubes C2 to C5) to provide the working calibrators as indicated in table 1 below. Tube C6 constitutes the assay blank and contains 1x diluent buffer only.

Table 1: Preparation of working calibration curve

Tube No.	CRP Concentration	Volume of calibrator	Volume of 1x diluent buffer	Serial dilution
C1 (Top calibrator)	120ng/ml	250µl	-	-
C2	60ng/ml	250µl	250µl	-
C3	30ng/ml	-	250µl	250µl of C2
C4	15ng/ml	-	250µl	250µl of C3
C5	7.5ng/ml	-	250µl	250µl of C4
C6 (Blank)	Blank		250µl	-

The range provided represents a CRP concentration of 3.75 – 60ug/ml when sample dilution of 1:500 is taken into account.

Procedure

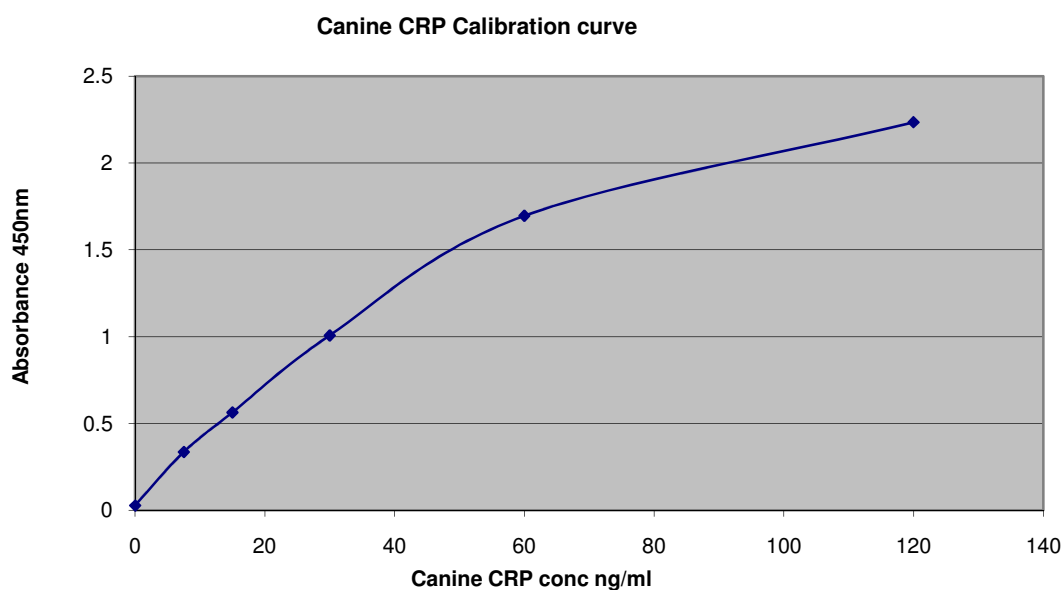
1. Determine the number of 8-well strips needed for the assay. Re-bag extra strips, seal bag and store desiccated in a refrigerator.
2. Add **100µl** of the diluted sample or calibrator, in duplicate, to each well.
3. Cover the plate with a dust cover and incubate the plate for **15 minutes at 37°C**.
4. After incubation aspirate or decant and wash the plate four times with diluted wash buffer. After the last wash tap the plate dry on absorbent paper.
5. Add **100µl** of conjugate to each of the wells.
6. Cover the plate with a dust cover and incubate the plate for **15 minutes at 37°C**.
7. After incubation aspirate or decant and wash the plate four times with diluted wash buffer. After the last wash tap the plate dry on absorbent paper.
8. Add **100µl** of TMB Substrate.
9. Cover the plate with a dust cover and incubate the plate for **15 minutes at room temperature**.
10. Add **100µl** of stop solution and tap gently to mix.
11. Read the absorbance of each well at 450nm using 630nm as a reference.

Interpretation of Test Results

1. Calculate the mean absorbance for each sample, control or calibrator.
2. Plot the absorbance of the calibrators against the calibrator concentration on **standard or semi-logarithmic** graph paper. (If necessary, the background absorbance for the 0ng/ml may be subtracted from each of the data points, including the calibrators, unknowns and controls prior to plotting). Draw the best smooth curve through these points to construct the calibration curve. Determine the concentrations of the test samples and controls from the calibration curve by multiplying the interpolated value by the appropriate dilution factor. Samples that have a signal greater than the top calibrator, or fall on the non-linear part of the curve, should be further diluted in diluent buffer and re-analysed.

Typical Data

An example of a typical calibration curve is represented below. This should not be used in the determination of canine CRP



Assay Reproducibility

Two samples containing medium and low levels of Canine CRP were run in independent assays. To determine inter assay reproducibility the mean and the coefficient of variation (%CV) were calculated.

Inter assay variation

Control	Level 1	Level 2
n	32	32
Mean (µg/ml)	14.9	34.6
Standard Deviation	1.2	2.7
%CV	8.2%	7.8%

Two samples containing medium and low levels of Canine CRP were tested in a single assay. To establish intra assay reproducibility the mean and the coefficient of variation (%CV) were calculated.

Intra assay Variation

Control	Level 1	Level 2
n	16	16
Mean (µg/ml)	15.4	35.8
Standard Deviation	1.1	2.3
%CV	6.9%	6.5%

Limitations of Test

The use of this test for the analysis of plasma samples has not been fully investigated. Haemolysed or lipaemic samples should not be used in the test.

Other “PHASE” acute phase assays available from Tridelta:

TP-801	Haptoglobin	Multispecies, colormetric, rapid assay, may be used in a manual method or on a large range of autoanalysers.
TP-801-Cal	Haptoglobin	Haptoglobin <i>calibrator</i> (2mg/ml) for Haptoglobin kit (TP-801)
TP-801-Con	Haptoglobin	Haptoglobin <i>controls</i> for Haptoglobin kit (TP-801)
TP-802	Serum Amyloid A	EIA Multispecies Serum Amyloid A immunoassay.
TP-802-Con	Multispecies SAA	Multispecies SAA <i>control</i> for Multispecies SAA kit (TP-802)
TP-807	Milk MAA	EIA Amyloid A specific for milk.
TP-802M	Murine SAA	EIA Serum Amyloid A assay specific for mouse
TA-901	CRP-Porcine	EIA C-reactive Protein assay specific for Porcine
TP-803-Con	Canine CRP	Canine CRP <i>controls</i> for Canine CRP EIA kit (TP-803)

Contact details follow:

Tridelta Development Limited

Unit 7, Block F,
Maynooth Business Campus
Maynooth
Co. Kildare
Ireland.

Tel: +353 1 6290635
Fax: +353 1 6290687

e-mail: general@trideltaltd.com
Web page: www.trideltaltd.com

CR015-01-10(2)