"PHASE"™ Porcine CRP Assay Cat. No. TA-901

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 pigs.

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 porcine CRP kit is a solid phase sandwich Immunoassay. Samples, including standards of known CRP content, bind to coated microwells. After washing to remove any unbound material the HRP labeled Anti-porcine-CRP antibody is added to each well. The immobilized antibody will bind specifically to any CRP in the 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

1.	Coated microplate	1 x 96 well plate
2.	C-Reactive protein standard	1 x 1.5ml (2x concentrate)
3.	Standard/sample diluent	1 x 50ml (10x concentrate)
4.	Anti-porcine 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 H_2O.
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 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 and Reagent 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^{o}C for up to 24 hours or stored frozen at –20^{o}C for longer periods without loss of CRP. It is important that all refrigerated samples are brought to room temperature and mixed to insure accurate determination of the CRP concentration.
All samples should be diluted 1:100 in sample dilution buffer prior to assay by addition of 10ul of sample to 1.0 ml dilution buffer.

Do not use grossly haemolysed or lipaemic samples.

Diluent Buffer

Dilute 1 volume of diluent buffer concentrate (10x) with 9 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^{o}C.

Wash Buffer

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^{o}C.

Standard Preparation

Table 1: Preparation of working standard curve

Tube No.	Concentration	Volume of standard	Volume of 1x diluent	Serial dilution
1	1500 ng/ml	250µl	250µl	-
2	750 ng/ml	-	250µl	250µl of 1
3	375 ng/ml	-	250µl	250µl of 2
4	187.5ng/ml	-	250µl	250µl of 3
5	93.75ng/ml	-	250µl	250µl of 4
6	46.9ng/ml	-	250µl	250µl of 5
7	Blank		250µl	-

Procedure

1. Determine the number of 8-well strips needed for the assay. Re-bag extra strips, seal bag and store in a refrigerator.
2. Add **100µl** of the diluted sample or standard, 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 plate 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 standard.
2. Plot the absorbance of the standards against the concentration of each standard. (If necessary, the background absorbance for the 0ng/ml may be subtracted from each of the data points, including the standards, unknowns and controls prior to plotting). Draw the best smooth curve through these points to construct the standard curve.
   Determine the concentrations of the test samples and controls from the standard curve by multiplying the interpolated value by the appropriate dilution factor. Samples that have a signal greater than the highest standard should be further diluted in diluent buffer and re-analysed.

Limitations of Test

Do not use grossly haemolysed or lipaemic samples.

Other "PHASE" acute phase assays available from Tridelta:

TP-801	Haptoglobin	Colormetric rapid test that can be used in a manual method or on a wide range of auto analysers. (Settings for some popular analysers available on request)
TP-801-Cal	Haptoglobin	Haptoglobin *calibrator* (2mg/ml) for Haptoglobin kit (TP-801).
TP-801-Con	Haptoglobin	Haptoglobin *controls* for Haptoglobin kit (TP-801).
TP-803	CRP-Canine	EIA C-reactive Protein assay specific for canine.
TP-803-Con	CRP-Canine	Canine CRP *controls* for Canine CRP EIA kits (TP-803).
TA-901	CRP-Porcine	EIA C- reactive Protein assay specific for porcine.
TP-802M	Murine SAA	EIA Serum Amyloid A assay specific for mouse.
TP-802M-CON	Murine SAA	M-SAA *controls* for Murine SAA Kit (TP-802M).
TP-807	Milk MAA	EIA Amyloid A specific for milk.
TP-802-CON	Multi-species SAA	SAA *controls* for Multi-species SAA Kit (TP-802).

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