DRG® CRP (rat) ELISA (EIA-4695)

Revised 26 July 2011 rm (Vers. 3.1) **For Veterinary Use Only**

Please use only the valid version of the package insert provided with the kit.

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

1 INTENDED USE

The DRG CRP (rat) ELISA is intended for the detection of rat C-reactive protein (CRP) in rat serum.

C-reactive protein is an acute-phase protein produced by the liver in conditions of inflammation, bacterial infection, or tissue trauma.

2 PRINCIPLE OF THE TEST

Rat sera for testing are diluted to 1:4,000 and allowed to react with antibodies coated on specially treated micro-wells. After appropriate incubation, the wells are washed to remove unreacted serum proteins, and an enzyme-labeled rabbit anti rat CRP (conjugate) is then added to react with and tag the antigen antibody complexes. Following another incubation period, the wells are again washed to remove unreacted conjugate. A urea peroxide substrate with TMB as chromogen is added to start color development. Development of a blue color indicates a positive reaction while negative reactions appear colorless or with a trace of blue. The reaction is interrupted with a stop solution that turns the blue positive reactions to yellow. Negative reactions remain colorless or with a hint of yellow. Color intensity (absorbance) is read at a wavelength of 450 nm on a spectrophotometer or ELISA reader. Semi-quantification of absorbance can be accomplished by the use of a standard curve generated by measuring two-fold dilutions of the standard provided.

3 MATERIALS SUPPLIED

The CRP (rat) ELISA kit supplies sufficient materials for 96 determinations.

1. CRP ELISA **microplate**
   96-well plate containing an affinity purified rabbit anti-rat CRP-IgG and packaged with desiccant, ready to use.
2. **Conjugate (100X)** 0.13 mL
   Concentrated affinity purified horseradish peroxidase (HRP) labeled rabbit anti rat CRP-IgG with stabilizers and a preservative.
   Protect from light.
3. CRP **Standard**, 1.33 mg/mL **(10X)**, 0.25 mL
   Rat serum with elevated CRP concentration.
   Serially dilute in three-fold dilutions four times, diluting the provided Standard 1:10 for the first standard.
4. **Wash Buffer**, 1 packet
   Tris buffer with Tween 20,
   pH 7.4 and 0.05% Tween 20 when reconstituted to 1 L with distilled water.

5. **TMB Substrate**, 12 mL
   A solution containing urea peroxide and 3,3', 5,5'-tetramethylbenzidine (TMB) supplied in a protective opaque bottle. Ready to use.
   Protect from light. Non-*carcinogenic*.
6. **Stop Solution**, 12 mL
   Diluted phosphoric acid. Ready to use.

4 MATERIAL REQUIRED BUT NOT SUPPLIED

1. Distilled or deionized (purified) water
2. Clean 250 mL or 500 mL wash bottle for wash buffer.
3. Test tubes or microtiter plate for preparing standard dilutions.
4. Precision pipette(s) (2 µL to 1000 µL) for making and delivering dilutions.
5. Adhesive cover for microplates.
6. ELISA reader equipped with a 450 nm filter. A program for data reduction would be helpful.

5 PREPARATION AND STORAGE OF REAGENTS

CRP (rat) ELISA components should be stored at 2-8°C. Bring them to room temperature (20-25°C) before opening bottles and plate pouches.

Diluted conjugate remaining after use should be discarded.

TMB substrate and stop solution are also stable at room temperature.

6 PRECAUTIONS

1. DO NOT INTERCHANGE COMPONENTS BETWEEN KITS AND DIFFERENT LOTS OF THE SAME TEST.
2. The standard serum and conjugate have not been screened for infectious agents. Since no testing can assure the absence of infectious agents, however, these reagents, as well as the serum specimens and equipment coming in contact with these specimens, should be handled with good laboratory practices to avoid skin contact and ingestion.
3. Do not use components past expiration date.
4. HRP-labeled conjugate and TMB-substrate are photosensitive and are packaged in a protective opaque bottle. Store in the dark and return to storage after use.

7 SPECIMEN COLLECTION AND PREPARATION

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 °C - 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 colder is recommended.

8 ASSAY PROCEDURE

8.1 Procedural Notes

IMPORTANT: Bring kit components to room temperature (20-25°C) before opening bottles and plate pouches. Allow at least 30 minutes for this process.

8.2 Test Procedure

1. Prepare wash buffer by adding 1 packet of powder to 1L of distilled water.
2. **Prepare the standards** as follows:
   Standard #1 = 133 ng/mL:
   Dilute provided standard 1:10, e.g. 1 unit of standard plus 9 units of wash buffer.
   Standard #2 = 44.5 ng/mL:
   Dilute Standard #1 three-fold, e.g. 1 unit of standard #1 plus 2 units of wash buffer.
   Standards #3 (14.8 ng/mL), and Standard #4 (4.9 ng/mL) standard #5 (1.6 ng/mL) are prepared by serial three-fold dilutions following standard #2.

Please consider the following dilution scheme as a guide

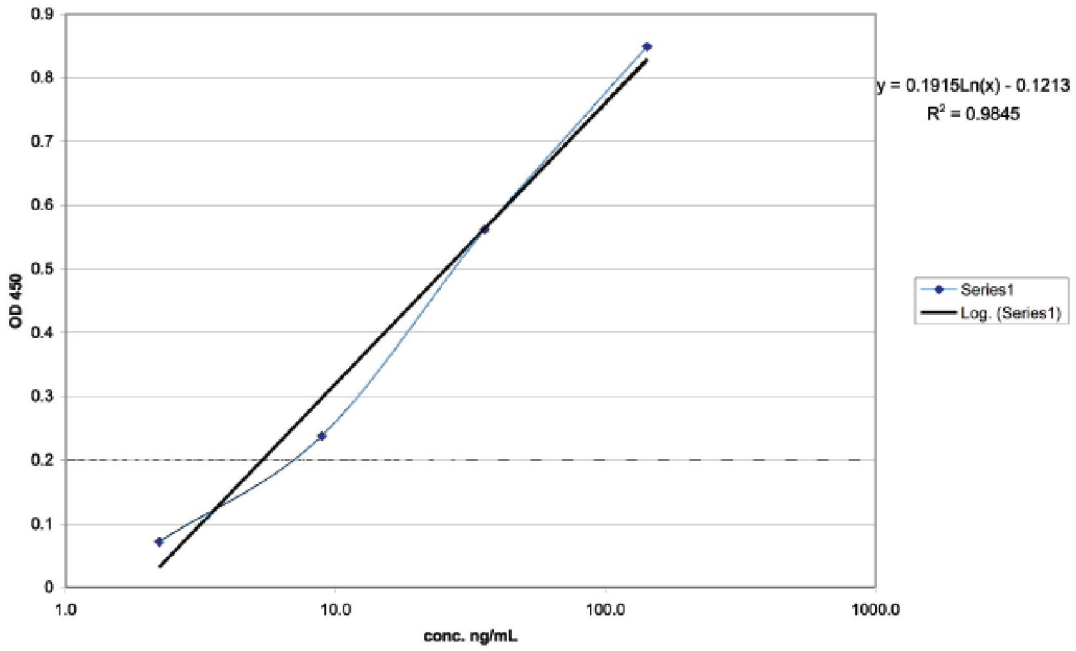
Standard #	Concentration	Volume Transferred	Diluent Volume	Total Volume	Final Volume
					(after dilutions)
1	133.3 ng/mL	18 µL	162 µL	180 µL	120 µL
2	44.5 ng/mL	60 µL	120 µL	180 µL	120 µL
3	14.8 ng/mL	60 µL	120 µL	180 µL	120 µL
4	4.9 ng/mL	60 µL	120 µL	180 µL	180 µL
5	1.6 ng/mL	60 µL	120 µL	180 µL	360 µL

3. **Sample preparation at 1:4,000**:
   a) First, dilute each serum sample 1:1,000 as follows:
   into a dilution vial, add 2 mL of wash buffer. To this, add 2 µL of serum.
   b) Then, dilute 1:4 by adding 1 part of a 1:1,000 sample to 3 parts wash buffer.
   e.g. 100 µL sample dilution to 300 µL buffer.
4. Add 100 µL to each well and **incubate at ambient temperature for 30 minutes**.
   Record the location for later reference.
5. Wash plates 4 - 5 times with a gentle stream of wash buffer from a wash bottle or a plate washer.
   Tap plates on a stack of absorbent paper towels to remove residual buffer.
6. Dilute stock conjugate (100x) to the desired working dilution (1x) with the Tris buffer,
   e.g. to 5 mL buffer, add 50 µL stock conjugate.
7. To each micro well, add 100 µL of conjugate.

8. Cover plate and **incubate for 30 minutes** at ambient temperature (20-25°C).
9. Wash plate as in step 5.
10. To each microwell, add 100 µL TMB/substrate solution and allow reaction to proceed at ambient temperature for **5 - 10 minutes**. Cover to avoid direct light. A blue color indicates a positive reaction.
11. Stop reaction by adding 100 µL of Stop solution to each well. Reaction mixture turns from blue to yellow.
12. Read absorbance (OD) on a microplate reader equipped with a 450 nm filter. A differential filter of 630 nm can also be used. Construct standard curve and read off values for patient samples or unknowns.
    Multiply values by 4,000 or other dilution factor to get actual serum concentration in ng/mL.
    Results can be expressed in µg /mL by dividing results by 1000.

9 RESULTS

TYPICAL CALIBRATION CURVE



Rat CRP (ng/mL)

Standard Curve used in the measurement of rat CRP in serum

10 LIMITATIONS

Lipemic sera may interfere with specific antibody reaction.

11 QUALITY CONTROL

Routinely run at least two controls each giving values at the top or bottom regions of the standard curve respectively. 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. 1:16,000 and 1:64,000 to obtain more accurate results.

12 REFERENCES/ LITERATURE

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