



*Manufactured for:*

**IBL-America, Inc.**

**RAT C-REACTIVE PROTEIN**

**FOR RESEARCH USE ONLY.**

**NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

**CAT NO. IB66103**

### **INTENDED USE**

The IBL-America C-reactive protein assay is a sandwich-based enzyme linked immunoassay intended for the detection of rat C-reactive protein (CRP) in rat serum or plasma. For research use only, not for use in diagnostic procedures.

### **PRINCIPLE OF THE TEST**

The Rat C-Reactive Protein Assay is species specific and provides a quantitative assessment of CRP levels in rat serum or plasma. The Rat 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-rat 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 rat CRP in the sample. Therefore, the greater the intensity of the blue color, the higher the rat 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	<b>Antibody Coated Microwell Plate</b>		96 wells (12 eight well strips) in a microwell holder coated with pneumococcal C-polysaccharide, <i>Ready-to-Use</i> .
1 X Vial	<b>Rat CRP Standard (10X)</b>	Green Cap	0.25ml of rat serum with elevated CRP concentration at 1.333 $\mu$ g/mL. Dilute standard stock 10X in Tris-T followed by three 3-fold serial dilutions to prepare standards at the following concentrations: 133.3, 44.4, 14.8, 4.9 and 0 ng/mL.
1 X Vial	<b>Anti-Rat CRP Conjugate (100X)</b>	Amber Cap	0.13mL of 100X anti-rat CRP-IgG protein conjugated to peroxidase in buffer with preservative. Dilute conjugate in Tris-T to 1X prior to use.
1 X Bottle	<b>Substrate Reagent</b>	Blue Cap	12m stabilized tetramethylbenzidine (TMB), <i>Ready-to-Use</i> .
1 X Bottle	<b>Stop Solution</b>	Red Cap	12mL Acidic Solution, <i>Ready-to-Use</i> .
1 X Pouch	<b>Washing Buffer</b>		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: 2 $\mu$ L to 1000  $\mu$ L
- Adhesive cover for microplate
- Microplate reader with 450nm filter

## **PRECAUTIONS**

1. Bring all reagents to room temperature (19<sup>o</sup>-27<sup>o</sup>C) before use.
2. Store reagents at 2<sup>o</sup>-8<sup>o</sup>C, 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 sample 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 rat serum proteins. Wear protective gloves and safety glasses when using this kit.
9. Dispose of all materials, containers, and devices in the appropriate receptacle after use.
10. 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**

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 colder is recommended.

Upon specimen collection, dilute the sample 1:1000 in Tris-Tween wash buffer (i.e. add 2µL of sample to 2mL of wash buffer). Additionally, dilute the 1:1000 sample 1:4 in Tris-Tween wash buffer (i.e. add 100µL sample to 300µL of wash buffer). The final dilution for use in calculation is 1:4000.

## **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 133.3ng/mL standard. Prepare remaining standards by serially diluting standards 3-fold three times to yield 44.4, 14.8, and 4.9ng/mL. Use Tris-Tween wash buffer for 0.0ng/mL standard. Consider the following dilution scheme as a guide:

<b>Standard Concentration (ng/mL)</b>	<b>Tris-Tween Volume</b>	<b>Volume Transferred</b>	<b>Total Volume</b>	<b>Final Volume *After Serial Dilution</b>
133.3ng/mL	162µL	18µL	180µL	120µL
44.5ng/mL	120µL	60µL	180µL	120µL
14.8ng/mL	120µL	60µL	180µL	120µL
4.9ng/mL	120µL	60µL	180µL	180µL

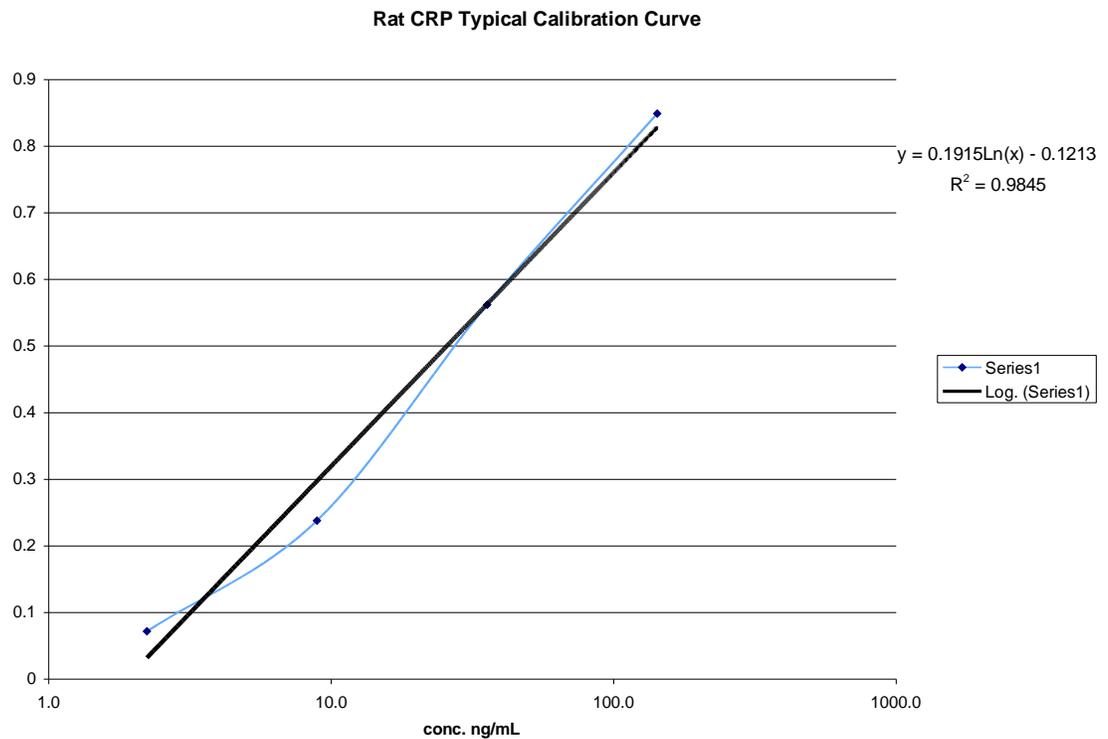
3. Using a new pipette tip for each, add 100µL of each standard and prepared sample into the appropriate wells. Samples and standards can be run in duplicate if desired. Incubate at room temperature for 30 minutes.
4. Decant the contents from the microwells into a discard basin. Wash the microwells by 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 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 concentrations 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**

Below is an example from a typical standard curve.



### **Intra-assay reproducibility**

A typical example of the Rat CRP Assay run in 12 replicates yielded the following within assay variation:

<b>Standard (ng/mL)</b>	<b>%CV</b>
133.3	8%
44.4	5%
14.8	5%
4.9	4%

**Inter-assay reproducibility**

The Rat CRP Assay exhibited the following variability with two different kit lots performed 12 times.

Standard (ng/mL)	%CV
133.3	7%
44.4	7%
14.8	4%
4.9	5%

**Limitations**

Lipemic sera may interfere with specific antibody reaction.

The expected range of CRP from healthy rats in sera is <300µg/mL CRP. Similar results are expected in plasma. Data on urine and other fluids are not available.

**Limit of Detection**

The lower and upper limit of quantification is 4.9 and 133.3 ng/mL, respectively.

**Sensitivity**

The Rat CRP Assay is designed to detect elevated levels of CRP. The following data was produced to generate data on the sensitivity of the assay and may be useful in research applications where sensitivity parameters need to be defined (n=11).

Mean OD	Standard Deviation	Detection Limit (ng/mL)
0.056	0.007	2.5

**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., 1:16,000 or 1:64,000) to obtain more accurate results.

**Cross-Reactivity**

Species	# of Runs	% Cross-reactivity
Mouse	10	100%
Canine	15	0%
Human	6	0%

## **REFERENCES**

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