



IBL-America, INC.
RAT C-REACTIVE PROTEIN
IB66103
FOR RESEARCH USE ONLY

INTENDED USE

The IBL-America C-reactive protein assay is intended for the detection and quantification of rat C-reactive protein (CRP) in rat serum.

CLINICAL RELEVANCE

C-reactive protein is synthesized in the liver following tissue damage caused by inflammation, infection, or trauma. It is considered to be an important acute phase marker in such conditions. It is an acute-phase protein and quantification of CRP is useful in determining inflammatory conditions difficult to diagnose and to monitor the patients' response to treatment.

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 450nm 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.

MATERIALS SUPPLIED

The IBL-America C-Reactive Protein 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) 0.12 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

Phosphate-buffered saline (PBS) with Tween 20, pH 7.4 and 0.05% Tween 20 when reconstituted to 1L 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.

6. Stop Solution, 12 mL

Diluted phosphoric acid. Ready to use.

MATERIAL REQUIRED BUT NOT SUPPLIED

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

PREPARATION AND STORAGE OF REAGENTS

IBL-America C-reactive protein kit 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.

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. 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.

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 are acceptable. If testing is to be delayed longer than 5 days, freezing the sample at -20°C or colder is recommended.

ASSAY PROCEDURE

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.

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 .tg/mL:** Dilute provided standard 1:10 ,e.g. 1 unit of standard plus 9 units of wash buffer.
 - **Standard #2 = 44.5 .tg/mL:** Dilute Standard #1 three-fold, e.g. 1 unit of standard #1 plus 2 units of wash buffer.
 - **Standards #3 (14.8 .tg/mL), and standard #4 (4.9 .tg/mL)** are prepared by serial three-fold dilutions following standard #2.
 -

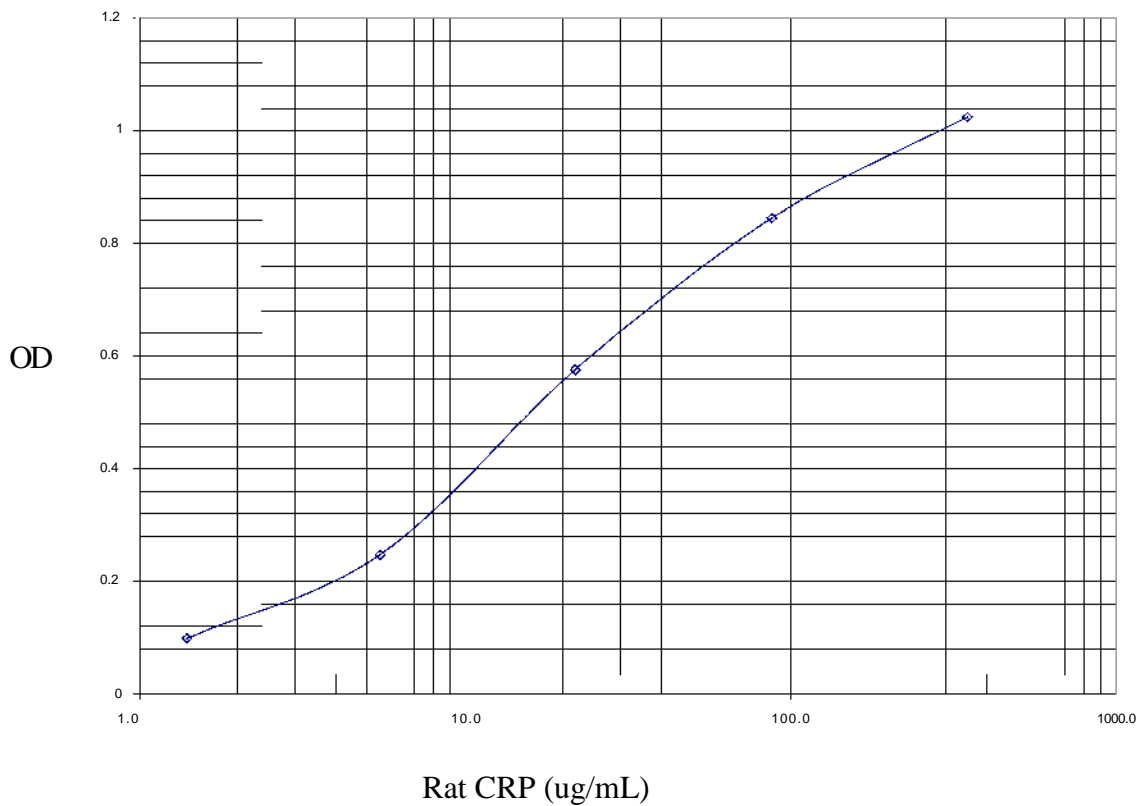
Please consider the following dilution scheme as a guide

Standard #	Concentration	Volume Transferred	Diluent Volume	Total Volume	Final Volume
1	133.3.tg/mL	18.tL	162.tL	180.tL	(after dilutions) 120.tL
2	44.5 .tg/mL	60 .tL	120 .tL	180 .tL	120 .tL
3	14.8 .tg/mL	60 .tL	120 .tL	180 .tL	120 .tL
4	4.9 .tg/mL	60 .tL	120 .tL	180 .tL	180 .tL

3. Sample preparation at 1:4,000: a) First, dilute each serum sample 1:1,000 as follows: into a dilution vial, add 2mL of wash buffer. To this, add 2uL of serum. b) Then, dilute 1:4 by adding 1 part of a 1:1,000 sample to 3 parts wash [buffer. eg.](#) 100 .tL sample dilution to 300.tL buffer.
4. Add 100ul 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 multi-channel micropipette or plate washer. Dispense approximately 300ul into each microwell. 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 PBS-T buffer, e.g. to 5 mL buffer, add 50uL stock conjugate.
7. To each microwell, add 100ul 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 100uL TMB/substrate solution and allow reaction to proceed at ambient temperature for 5 - 10 minutes. A blue color indicates a positive reaction.
11. Stop reaction by adding 1 00uL of Stop solution to each well. Reaction mixture turns from blue to yellow.
12. Read absorbance (OD) on a microplate reader equipped with a 450nm 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 to get actual serum concentration.

RESULTS

TYPICAL CALIBRATION CURVE



Standard Curve used in the measurement of rat CRP in serum

LIMITATIONS

Lipemic sera may interfere with specific antibody reaction.

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.

EXPECTED VALUES

A study performed on over 200 sera from healthy rats showed a range of 200 – 600 ug/mL CRP. In serum. Similar results are expected on plasma. Data on urine and other fluids is not available.

PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY

Inter-assay reproducibility (2 plate lots)	
tested 12 times	CV (%)
ug/mL	
125.0	7
62.5	7
31.3	4
15.6	5
7.8	3
Intra-assay reproducibility (tested 12 times)	
ug/mL	CV (%)
125.0	8
62.5	5
31.3	5
15.6	4
7.8	4

SENSITIVITY

The IBL-America 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 maybe useful in research applications where sensitivity parameters need to be defined.

Assay

Sensitivity n=1 1

Sample	Mean [OD]	Standard Variation	Detection Limit [ng/ml]
1	0.056	0.007	2.5 ng/ml

CROSS REACTIVITY

Canine CRP	0/15	0%
------------	------	----

Manufactured For

Immuno-Biological Laboratories
 8201 Central Ave. NE, Suite P,
 Minneapolis, Minnesota 55432 USA
 Tel: (763)-780-2955
 FAX: (763)-780-2988
[Email: ibl@ibl-america.com](mailto:ibl@ibl-america.com)

