Technical Data Sheet

Rat C-Reactive Protein [CRP] ELISA Kit

Product Information

Material Number: **557825**

Description

Materials Provided

Capture antibody coated microplate

One plate of 96 breakable wells coated with a rabbit anti-rat CRP antibody

Detection antibody/Enzyme Conjugate (100x)

120 µL of concentrated horseradish peroxidase (HRP) conjugated to a rabbit anti-rat CRP antibody containing stabilizers and preservative. Protect from light.

Standard (10x)

250 µL of rat serum with elevated levels of CRP.

Wash Buffer

One packet of powdered phosphate-buffered saline (PBS) with 0.05% Tween-20. Reconstitute with 1L distilled water.

TMB Substrate

A 12 mL solution containing 3,3',5,5'-tetramethylbenzidine (TMB) supplied in a protective opaque bottle. Protect from light.

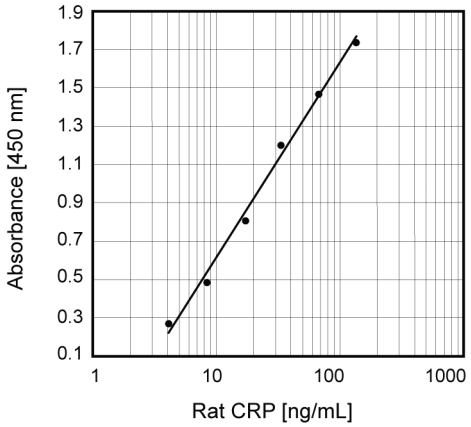
Stop Solution

12 mL of diluted phosphoric acid, ready to use.

The rat C-reactive protein (CRP) ELISA kit is designed for the detection and quantitation of rat CRP in rat serum. CRP is an acute-phase protein produced by the liver during conditions of inflammation, bacterial infection, or tissue trauma. Quantitation of CRP can be useful for the determination of inflammatory conditions that would be otherwise difficult to detect and monitor.

Principle of the Assay

The BD™ ELISA for rat CRP is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes an antibody specific for rat CRP coated on a 96-well plate. Standards or samples are added to the wells, and any rat CRP present binds to the immobilized antibody. After the appropriate incubation, wells are washed and a horseradish peroxidase conjugated anti-rat CRP is added to produce an antibody-antigen-antibody "sandwich". Following another incubation period, the wells are washed again and a substrate solution is added, which produces a blue color in direct proportion to the amount of CRP present in the initial sample (development of a blue color indicates a positive reaction while negative reactions appear colorless). The reaction is interrupted with the Stop Solution, which changes the color from blue to yellow (negative reactions remain colorless or faintly yellow). The color is then measured at a wavelength of 450 nm (absorbance) on a spectrophotometer or on an ELISA reader.



Preparation and Storage

Store undiluted at 4°C.

Kit components should be brought to room temperature (20-25°C) before opening bottles and plate pouches. Allow at least 30 minutes for this process. Do not use the kit after the expiration date.

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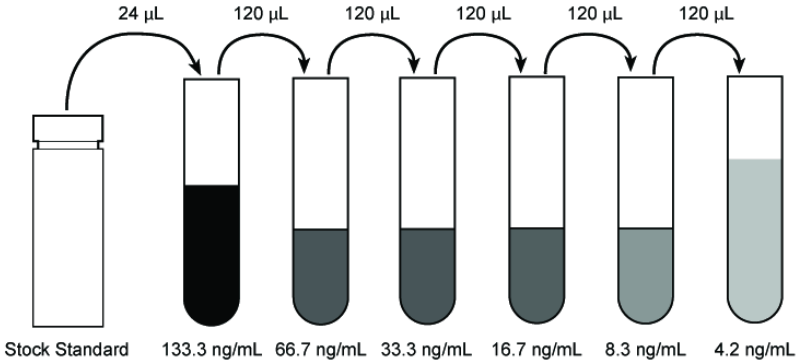

Standard #	Final concentration	Volume transferred	Diluent volume (wash buffer)	Total volume	Final volume (after dilutions)	Dilution factor
Stock (10x)	1333 ng/mL	NA	NA	NA	NA	NA
1	133.3 ng/mL	24 µL	216 µL	240 µL	120 µL	1:10
2	66.7 ng/mL	120 µL	120 µL	240 µL	120 µL	1:2
3	33.3 ng/mL	120 µL	120 µL	240 µL	120 µL	1:2
4	16.7 ng/mL	120 µL	120 µL	240 µL	120 µL	1:2
5	8.3 ng/mL	120 µL	120 µL	240 µL	120 µL	1:2
6	4.2 ng/mL	120 µL	120 µL	240 µL	240 µL	1:2

Application Notes

Application

ELISA	Tested During Development

Recommended Assay Procedure:

Specimen Collection and Handling:

Specimens should be clear, non-hemolyzed and non-lipemic. Blood samples should be collected using approved venipuncture techniques. Allow samples to clot and separate serum by centrifugation. Alternatively, use a serum separator tube (BD Vacutainer Cat.No. 366430) and allow samples to clot for 30 minutes, then centrifuge for 10 minutes at 1000 x g. Remove serum and assay immediately or store samples at ≤ -20°C. Avoid repeated freeze-thaws of the samples.

Additional Materials Required:

1. Distilled or deionized water
2. Graduated cylinder, one liter
3. Wash bottle or automated washer
4. Precision pipettes
5. Tubes or microtiter plate to prepare standard dilutions
6. Plate sealers or parafilm
7. Laboratory timer
8. Graph paper or automated data reduction software
9. Microplate reader capable of measuring absorbance at 450 nm

Assay Procedure:

1. Prepare Wash Buffer by dissolving 1 packet of powdered PBS in 1L of distilled water.
2. Prepare the CRP standard by diluting the provided 10x stock standard 1:10. Thereafter, perform 1:2 serial dilutions five times. See figure with dilution scheme. For example, to prepare standard for one well:
   Standard #1: Dilute the stock standard 1:10 (i.e. 24 µL of 10x stock standard mixed with 216 µL of Wash Buffer)
   Standard #2: Dilute Standard #1 1:2 (i.e. 120 µL of Standard #1 mixed with 120 µL of Wash Buffer)
   Standard #3: Dilute Standard #2 1:2 (i.e. 120 µL of Standard #2 mixed with 120 µL of Wash Buffer)
   Standard #4: Dilute Standard #3 1:2 (i.e. 120 µL of Standard #3 mixed with 120 µL of Wash Buffer)
   Standard #5: Dilute Standard #4 1:2 (i.e. 120 µL of Standard #4 mixed with 120 µL of Wash Buffer)
   Standard #6: Dilute Standard #5 1:2 (i.e. 120 µL of Standard #5 mixed with 120 µL of Wash Buffer)
3. Serum samples will likely need to be diluted before testing and a titration of the sample is strongly recommended. The suggested dilution for rat serum is 1:4000 (i.e. 2 µL of serum into 2 mL wash buffer for a 1:1000 dilution. Next, add 100 µL of the diluted serum and add into 300 µL wash buffer for a 1:4 dilution to give the final working dilution of 1:4000).
4. Add 100 µl to each well and incubate at room temperature for 30 minutes.
5. Wash the plate 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. Prepare a 1x working concentration of the detection antibody/enzyme conjugate by diluting the provided 100x detection antibody/enzyme conjugate 1:100 with Wash Buffer (e.g. add 50 µL of 100x detection antibody/enzyme conjugate to 5 mL of Wash Buffer).
7. To each microwell being testing, add 100 µl of the 1x detection antibody/enzyme conjugate.
8. Cover the plate and incubate for 30 minutes at room temperature.
9. Wash the plate as described in step 5.
10. Add 100 µL of TMB substrate solution and incubate 5-10 minutes at room temperature.
11. Stop reaction by adding 100 µL of Stop solution.
12. Read absorbance at 450 nm within 30 minutes of stopping the reaction. If wavelength correction is available, absorbance at 630 nm may be subtracted from absorbance at 450 nm.
13. The provided 10x stock standard represents pre-diluted serum. To determine sample CRP concentrations, multiply measured values by the dilution factor used (4000 if a 1:4000 dilution was performed) to account for the dilution during sample preparation.

Limitations of the Procedure:

1. The BD™ ELISA Rat CRP kit is intended for use as an integral unit. Do not mix reagents from different kit batches.
2. Samples that generate absorbance values higher than the standard curve should be diluted and retested. Occasionally, antigen excess may be encountered in serum with high CRP values. In this situation, all the available CRP may not have reacted with the detection antibody and testing the serum at higher dilutions (e.g. 1:8000, 1:16000 or 1:64000) may be necessary.
3. Interference by drug metabolites, soluble receptors, or other binding proteins in specimens has not been thoroughly investigated. The possibility of interference cannot be excluded.
4. Do not use components past the expiration date.

Handle all serum and plasma specimens in accordance with NCCLS guidelines for preventing transmission of blood-borne infections. The control serum has not been screened for infectious agents. Since no testing can assure the complete absence of infectious agents, serum specimens and equipment coming in contact with these specimens should be handled with good laboratory practices.

Warning: *PBS-T Wash Buffer Powder (component 51-915X001) contains 75.8% sodium chloride (w/w) and 1.9% (w/w) potassium chloride (w/w).*

Hazard statements

May be harmful if swallowed.

Precautionary statements

Call a POSION CENTER/doctor if you feel unwell.

Danger: *Stop Solution (component 51-946P001) contains 15.23% phosphoric acid (w/w).*

Hazard statements

Causes severe skin burns and eye damage.

Precautionary statements

Wear protective gloves / eye protection.
Wear protective clothing.
IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
Dispose of contents/container in accordance with local/regional/national/international regulations.

Product Notices

1. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Banerjee M, Tripathi LM, Srivastava VM, Puri A, Shukla R. Modulation of inflammatory mediators by ibuprofen and curcumin treatment during chronic inflammation in rat. *Immunopharmacol Immunotoxicol.* 2003; 25(2):213-224. (Biology)

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Nathan BR, Scheld WM. The potential roles of C-reactive protein and procalcitonin concentrations in the serum and cerebrospinal fluid in the diagnosis of bacterial meningitis. *Curr Clin Top Infect Dis.* 2002; 22:155-165. (Biology)

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