Rat C-Reactive Protein ELISA

Catalog Number: 88-7501
RUO: For Research Use Only. Not for use in diagnostic procedures.

Product Information

Contents: Rat C-Reactive Protein ELISA
REF **Catalog Number:** 88-7501
Sensitivity: 2 ng/mL
Standard Curve Range: 2-133 ng/mL

Temperature Limitation: Store at 2-8°C.
LOT **Batch Code:** Refer to vial
Use By: Refer to vial

Description

This rat C-reactive protein (CRP) kit contains all of the necessary buffers and reagents for performing enzyme-linked immunosorbent assay (ELISA). The ELISA set reagents have been optimized for the accurate and precise measurement of rat CRP in serum, plasma, urine, and tissue culture supernatant samples.

C-reactive protein (CRP) is a homopentameric protein belonging to the pentraxin family with a molecular weight of about 115 kDa. CRP is an acute-phase protein produced by hepatocytes in response to circulating IL-6, IL-1, and TNF alpha. It plays an important role in host defense as a pro-inflammatory mediator and activator of the complement pathway. Although normal circulation levels are low, they rise sharply within 48 hours of disease or trauma and stay elevated until the resolution of inflammation. Elevated levels of CRP are associated with many pathological states, including rheumatoid arthritis, tissue trauma, viral or bacterial infection, hepatitis, and some autoimmune conditions. CRP has also been shown to be a reliable indicator of increased risk of cardiovascular disease and post-operative complications.

Components

Pre-coated ELISA Plate
Detection Antibody. Pre-titrated, HRP-conjugated antibody
Standard. Protein for generating standard curve and calibrating samples
Tris Wash Buffer Powder
Substrate Solution. Tetramethylbenzidine (TMB) solution
Stop Solution

Applications Reported

The rat CRP ELISA kit is intended for the measurement of rat CRP in serum, plasma, urine, and tissue culture supernatant samples.

Special Notes

To ensure optimal performance from this ELISA kit, please only use the components included in the kit. Exchanging of components is not recommended as a change in signal may occur.

References

Patel VB, Robbins MA, and Topol EJ. C-reactive protein: A ‘golden marker’ for inflammation and coronary artery disease. Cleve Clin J Med 2001 Jun; 68(6): 521-524, 527-534

Hirschfield GM and Pepys MB. C-reactive protein and cardiovascular disease: new insights from an old molecule. QJM 2003 Nov; 96(11): 793-807

Dhingra R, Gona P, Nam BH, D’Agostino RB Sr, Wilson PB, Benjamin EJ, and O’Donnell CJ. C-reactive protein, inflammatory conditions, and cardiovascular disease risk. Am J Med 2007 Dec; 120(12): 1054-1062

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Rat C-Reactive Protein (CRP) ELISA Kit Protocol

Introduction

The rat C-reactive protein (CRP) ELISA kit is intended for the quantitative detection of rat CRP in serum, plasma, urine, and tissue culture supernatant samples. C-reactive protein is an acute-phase protein produced by hepatocytes and is present at elevated levels following tissue trauma, bacterial infection, or conditions of inflammation.

Rat CRP present in the samples and standards binds to the anti-Rat-CRP antibodies adsorbed to the plate. Following incubation, a wash is performed to remove any unbound particles and an HRP-conjugated antibody against a distinct epitope of rat CRP is added to the microwell plate. After a second incubation period and wash, a peroxidase substrate is added. The development of blue color is in proportion to the amount of soluble CRP present in the sample. The reaction can be stopped with an acid solution that turns the liquid from blue to yellow. Color intensity can be determined reading the absorbance at 450 nm on a spectrophotometer. A dilution series of standards at known concentrations can be used to plot a curve and quantify the amount of rat CRP present in the samples

General Notes

Sample Preparation:

1. Allow blood samples to clot and separate serum by centrifugation. Assay samples immediately or store at ≤-20°C. Avoid repeated freeze/thaw cycles. Grossly hemolyzed or lipemic samples are not suitable for evaluation in this assay.
2. Plasma collected from blood drawn in heparin, EDTA, or ACD-containing tubes is also acceptable for evaluation in this assay.
3. Serum and plasma samples must be diluted at least 1:4,000 prior to assay.
4. Samples other than serum or plasma should be used at a higher concentration. A recommended starting point is a 1:2 dilution, with increasing dilutions as the user sees fit.

Storage of Reagents:

The components of this ELISA kit should be stored at 2-8°C and brought to room temperature prior to opening bottles or plate pouches. Allow at least 30 minutes for this process. Any diluted reagents remaining after use should be discarded. TMB substrate and stop solution are also stable at room temperature.

Precautions:

1. To ensure optimal results, only use the components included in this set. Exchanging of components is not recommended as a change in signal may occur.
2. Do not use components past expiration date.
3. The standard serum and conjugate have not been screened for infectious agents. All reagents, samples, and any equipment that comes in contact with them should be handled using good safety practices to prevent skin contact or ingestion.
4. The HRP-conjugate and TMB substrate included in this kit are photosensitive and should be stored in the dark when not in use.

Protocol

Materials

- Rat C-Reactive Protein ELISA microwell plate
- Anti-Rat C-Reactive Protein, HRP-conjugated, 0.13 mL
- Rat C-Reactive Protein Standard, 0.25 mL
- Wash puffer powder, 1 packet
- TMB substrate, 12 mL
- Stop solution, 12 mL

Other Materials Required

- Distilled or deionized water

- Wash bottle
- Test tubes
- Adhesive microplate covers

Instruments

- Precision pipette(s), 2 µL to 1000 µL
- ELISA plate reader equipped with a 450 nm filter
- ELISA plate washer

Experiment Duration

- 2 hour incubation at room temperature of standards and samples
- 1 hour incubation at room temperature of HRP-conjugate
- 5-10 minute substrate color development

Experimental Procedure

1. Prepare wash buffer by adding 1 packet of powder to 1 L of distilled or deionized water.
2. Pipette 450 µL of wash buffer into a test tube. Add 50 µL of the provided standard (1:10 dilution) to create the top standard, 133 ng/mL.
3. Pipette 250 µL of wash buffer into each of six tubes. Add 250 µL of the top standard dilution from Step 2 and mix well. Continue the 2-fold dilution series to create the standard curve. The wash buffer alone serves as the zero standard, or blank.
4. Dilute samples as necessary in wash buffer. Serum and plasma samples should be diluted at least 1:4,000 prior to assay. Other sample types should be evaluated at a higher concentration as determined by the user.
5. Add 100 µL of standard or sample per well of the microwell plate. Record locations of each addition for later reference. Seal plate with an adhesive strip and incubate at room temperature for 2 hours.

   Note: All samples and standards should be evaluated in duplicate or triplicate to ensure accuracy of results.
6. Wash the plate 5 times with wash buffer using an ELISA plate washer or a wash bottle. Following the final wash, invert and tap the plate on a paper towel to remove residual buffer.
7. Dilute the HRP-conjugated antibody 1:100 in wash buffer. For example, to 9.9 mL of wash buffer, add 100 µL of stock conjugate.
8. Add 100 µL of the diluted conjugate to each well of the plate. Seal and incubate for 1 hour at room temperature.
9. Repeat wash as in Step 6.
10. To each well, add 100 µL of the TMB substrate solution. A blue color indicates a positive reaction. Allow reaction to proceed at room temperature for 5-10 minutes.
11. Stop reaction by adding 100 µL of stop solution per well. The reaction mixture should turn to yellow.
12. Read the absorbance (O.D.) on an ELISA plate reader equipped with a 450 nm filter. If wavelength correction is available, set to 570 nm. Readings made without wavelength correction may be higher and less accurate.
13. Create a standard curve by using software capable of creating a four-parameter logistic (4-PL) fit. If this is not available, a suitable alternative is to plot the log of the standard concentrations versus the log of mean O.D. and determining the line of best fit by regression analysis.
14. Use the standard curve to determine the amount of rat CRP present in the samples. The concentration read from the curve should be multiplied by the dilution factor for any diluted samples.

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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