Rat C-Reactive Protein (CRP) ELISA Kit (96-well)

Cat. No. CYT294

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

The rat C-Reactive Protein (CRP) kit is a double polyclonal antibody sandwich enzyme immunoassay (EIA), which measures rat CRP.

Test Principle

With the rat CRP ELISA, standards, quality controls and samples of sera are incubated in microtitration wells coated with polyclonal anti-rat CRP antibody. After a thorough wash, polyclonal anti-rat CRP antibody labelled with horseradish peroxidase (HRP) is added to the wells and incubated with the immobilized antibody-CRP complex. Following another washing step, the remaining HRP-conjugated antibody is allowed to react with the substrate and tetramethylbenzidine. The reaction is stopped by addition of acidic solution, and absorbance of the resulting yellow color product is measured spectrophotometrically at 450 nm. The absorbance is proportional to the concentration of CRP. A standard curve is constructed by plotting absorbance values versus CRP concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

Application

The rat C-reactive protein (CRP) kit is designed to measure the amount of rat CRP in serum or plasma. There are enough reagents included in this kit for one 96-well immuno-assay plate. We recommend running duplicate wells for samples and standards.

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Analytical Sensitivity and Detection Limits

Sensitivity:	2.5 ng/mL
Species reactivity:	Rat. No detectable reactivity with canine serum.
Intra-assay Variation:	± 8%
Inter-assay Variation:	± 7%

Kit Materials

1. Microwell Strips (96 wells), coated with Anti-rat CRP Antibody, vacuum sealed (Catalog No. CYT294a)
2. Conjugate Concentrate (100x) (Anti-rat CRP Antibody, Horseradish Peroxidase Conjugate), 0.13 mL (Catalog No. CYT294b)
3. Rat CRP Standard, 1.33 ug/mL (10x), 0.25 mL (Catalog No. CYT294c)
4. Substrate (TMB) Solution, 12 mL, Protect from light. Non-carcinogenic. (Catalog No. CYT294d)
5. Stop Solution (diluted phosphoric acid), 12 mL (Catalog No. CYT294e)
6. Wash Buffer, 1 packet (PBS with 0.05% Tween 20, pH 7.4 when reconstituted to 1 liter of distilled water) (Catalog Number CYT294f)

Materials Required But Not Provided

1. Test tubes for diluting samples
2. Precision pipettes to deliver 2-1000 µL
3. Microplate reader with 450 ± □10 nm filter
4. Software package facilitating data generation and analysis
5. Microtitration plate washer (optional) [Manual washing is possible but not preferable.]
6. Absorbent material for blotting the microwell plate
7. Clean 250-500 mL wash bottle for buffer
8. Adhesive cover for microplates
9. Deionized (distilled) water

Precautions

- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains urea peroxide. Wear gloves and eye protection when handling these reagents. In case of contact with the Stop Solution and the Substrate Solution wash skin thoroughly with water and seek medical attention, when necessary.
- 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.
- Wear gloves and laboratory coats when handling the kit.
- The materials must not be pipetted by mouth.
- Do not drink, eat or smoke in the areas where the kit is being handled.
- Reagents with different lot numbers should not be mixed.

Preparation of Reagents

All reagents need to be brought to room temperature prior to the assay.

Preparation of Wash Solution:

Add one Wash Buffer packet to 1,000 mL deionized (distilled) water.

Preparation of Standard:

Prepare standards as follows:

Standard #1, 133.3 ng/mL = Dilute provided standard 1:10 (e.g. 1 unit of standard to 9 units of Wash Buffer, 18 µL of standard to 162 µL of Wash Buffer).

Standard #2, 44.5 ng/mL = Dilute provided standard 1:3 (e.g. 1 unit of standard to 2 units of Wash Buffer, 60 µL of standard to 120 µL of Wash Buffer).

Standards #3, 14.8 ng/mL and Standard #4, 4.9 ng/mL = Prepare by serial three-fold dilutions following Standard #2.

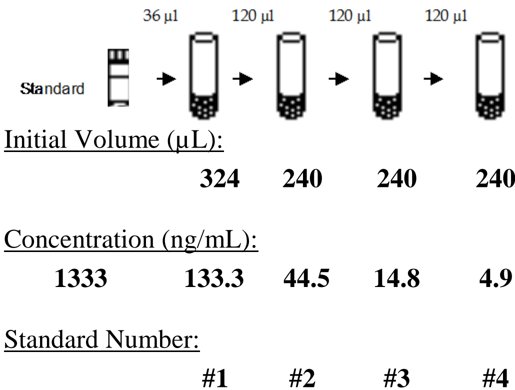

Figure 1: Serial Dilution of CRP Standard

Preparation of Conjugate:

Dilute stock conjugate (100x) to the desired concentration of 1x with Wash Buffer (e.g. add 50 µL of stock conjugate to 5 mL of buffer).

Preparation of Samples

Blood samples should be collected using approved venipuncture techniques by qualified personnel. Allow blood to clot and separate serum by centrifugation. Transfer serum aseptically to a tightly closing sterile container. Store at 2° to 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 samples at -20°C or colder is recommended.

Dilute samples 1:4,000 with Wash Buffer as follows: a) First dilute each sample 1:1,000 by adding 2 µL of sample to 2 mL of Wash Buffer. b) Then dilute 1:4 by adding 1 part of the 1:1,000 sample to 3 parts of Wash Buffer (eg 100 µL of sample to 300 µL of Wash Buffer). Do not store diluted samples.

Storage of Kit

- Store the kit at 2° to 8°C for up to 6 months after date of receipt or until expiration date.

Assay Instructions

1. Dispense 100 µL of the diluted Standards, controls and samples, in duplicate, into their designated wells.

   NOTE: A STANDARD CURVE MUST BE RUN AT EACH SETTING.

2. Incubate the plate at room temperature (20-25°C) for 30 minutes.
3. Wash the plate 4-5 times with Wash Solution (0.35 mL per well). Tap the plate on a stack of absorbent paper towels to remove residual buffer.
4. Add 100 µL of diluted Conjugate Solution.
5. Incubate the plate at room temperature (20-25°C) for 30 minutes.
6. Wash the plate as in step 3. Tap the plate on a stack of absorbent paper towels to remove residual buffer.
7. Add 100 µL of Substrate Solution. (Avoid exposing the microwell plate to direct sunlight. Covering the plate with e.g. aluminum foil is recommended.)
8. Incubate the plate for 5-10 minutes at room temperature (20-25°C). A blue color indicates a positive reaction.
9. Stop the color development by adding 100 µL of Stop Solution. The reaction mixture will turn from blue to yellow.
10. Determine the absorbance by reading the plate at 450 nm. A differential filter of 630 nm can also be used.

Calculation of Results

Construct standard curve and read off values for samples. Multiply concentrations in ng/mL by the dilution factor 4000 to obtain CRP concentrations in ng/mL in the original undiluted serum samples. Divide the values by 1000 to obtain the concentrations in ug/mL.

Limits of Assay

Lipemic sera may interfere with the 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 CRP may not have reacted with the conjugate. It is suggested that you test at a higher dilution, e.g. 1:16,000 to 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 μg/mL. Plasma samples are expected to give similar results.

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