

Rabbit C-reactive protein (CRP) ELISA Kit

to determine Rabbit C-reactive protein in Serum, Blood Plasma, Saliva, Urine, And Other Related Tissue Liquid Samples.

INSTRUCTION MANUAL

FOR ELISA KIT No: LTB6100EA



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INTENDED USE

This kit is used to determine the Rabbit C-reactive protein (CRP) in the sample of serum, blood plasma, saliva, urine, and other related biological liquid. For *in vitro* use only.

MANUAL VERSION 1.02

BACKGROUND

C-reactive protein encoded by CRP gene belongs to the pentraxin family. It is involved in several host defense related functions based on its ability to recognize foreign pathogens and damaged cells of the host and to initiate their elimination by interacting with humoral and cellular effector systems in the blood. Consequently, the level of C-reactive protein in plasma increases greatly during acute phase response to tissue injury, infection, or other inflammatory stimuli.

PRINCIPLE

Rabbit C-reactive protein (CRP) ELISA Kit employs a two-site sandwich ELISA to quantitate CRP in samples. An antibody specific for CRP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CRP present is bound by the immobilized antibody. After removing any unbound substances, HRP-Conjugate Rabbit CRP detection antibody is added to the wells. Following a wash to remove any unbound HRP reagent, a Chromogen solution is added to the wells and color develops in proportion to the amount of CRP bound in the initial step. The color development is stopped and the intensity of the color is measured.

MATERIALS SUPPLIED IN THIS KIT

Rabbit CRP microplate: 96 well polystyrene microplates (8 strips of 12 wells) coated with the antibody specific for Rabbit CRP.

Rabbit CRP standard: Rabbit CRP in a buffered protein, liquid.

Standard diluent: Diluent solution for reconstituted standard.

Sample diluent: Diluent solution for reconstituted samples.

HRP-Conjugate Rabbit CRP detection antibody: Antibody specific for Rabbit CRP, liquid.

Chromogen solution A: liquid.

Chromogen solution B: liquid.

Stop solution: liquid.

Wash buffer: 30X liquid.

Plate covers.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator
2. Microplate reader
3. Precision pipettes and tips
4. Distilled water
5. Disposable tubes for sample dilution
6. Absorbent paper

IMPORTANT NOTES

1. Before using, keep the kit outside and allow it to come to room temperature.
2. After breaking the seal of ELISA coated-plate, keep the unused strips in the zipper bag at 2-8 °C and use within one month.
3. Pipette tips and seal plate membrane should not be used more than once in order to avoid cross contamination.
4. All samples and all discard generated should be disposed as per local rules.
5. Reagents of different batches must not be mixed and should be used before their respective validity dates.
6. Substrate B is sensitive to light and therefore should not be exposed to light for too long.

PRECISION

Intra-assay Precision (Precision within an assay): Four samples of known

concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%) <9%.

Inter-assay Precision (Precision between assays): Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision.

Assays were performed by at least three technicians using two lots of components.

The CV (%) <11%.

Recovery: The recovery of Rabbit CRP spiked to different levels in samples throughout the range of the assay in various matrices was evaluated.

The recovery ranged from 98% to 116% with an overall mean recovery of 106%

Sensitivity: The minimum detectable dose (MDD) of Rabbit CRP is typically less than 5.0 µg/L. The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity: Rabbit C-reactive protein ELISA Kit can be used to measure Rabbit CRP in samples. Rabbit C-reactive protein ELISA Kit has high sensitivity and excellent specificity for detection of Rabbit CRP. No significant cross-reactivity or interference between Rabbit CRP and analogues was observed.

SAMPLE PREPARATION

1. Samples containing NaN₃ are not recommended for testing with ELISA as they may inhibit the activity of Horse Radish Peroxidase (HRP).
2. After extraction, experiment should be conducted immediately. Otherwise, keep

the sample at -20 °C. Avoid repeated freeze-thaw cycles.

3. Serum: Allow the sample to clot for 10-20 minutes at room temperature. Centrifuge (at 2000-3000 RCF) for 20 minutes. Collect the supernatant carefully. In case of storage, centrifugation should be performed again prior to use.
4. Blood plasma: During sample collection EDTA or sodium citrate should be used for anti-coagulation. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.
5. Urine: Collect the sample in a sterile tube. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow a similar procedure.
6. Cell culture supernatant: For secreted components, centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells by repeated freeze-thaw cycles to let out the inside components. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes and collect the supernatants carefully. In case of storage, centrifugation should be performed again prior to use.
7. Tissue sample: Incise tissue sample of interest and add few mls of PBS (pH 7.4). Freeze with liquid nitrogen immediately for later use. Thaw the sample and keep it at 2-8 °C. Add few mls of PBS (pH 7.4) and then homogenize the sample thoroughly by hand or by homogenizer. Centrifuge (at 2000-3000 RCF) for approximately 20 minutes. Collect the supernatants carefully. Aliquot and keep one for examination and freeze the others for later use.

ASSAY PROCEDURE

1. Dilution of standard solution: This kit contains a standard of known concentration, which could be diluted in small tubes by the end-user by following the instruction in the table below:

1200µg/L	Standard No.5	150µl Original Standard + 150µl Standard diluents
600µg/L	Standard No.4	150µl Standard No.5 + 150µl Standard diluents
300µg/L	Standard No.3	150µl Standard No.4 + 150µl Standard diluent
150µg/L	Standard No.2	150µl Standard No.3 + 150µl Standard diluent
75µg/L	Standard No.1	150µl Standard No.2 + 150µl Standard diluent



2. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the microplate.
3. Add standard: Set Standard wells, testing sample wells. Add diluted standard 50µl to standard well.
4. Add Sample: Add sample diluent 40 µl to testing sample well. Then add sample 10µl to testing sample well; for Blank well don't add anything.
5. Cover with a plate cover and incubate for 45 minutes at 37 °C.
6. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash buffer (250 µl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

7. Add 50 µl HRP-Conjugate to each well, except blank well.
8. Cover with a new adhesive strip. Incubate for 30 minutes at 37 °C.
9. Repeat the aspiration/wash process for five times as in step 5.
10. Add 50 µl chromogen solution A and 50 µl chromogen solution B to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.
11. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

PROTOCOL SUMMARY

Prepare reagents, samples and standards.



Add prepared samples and standards in respective wells together and incubate for 45 minutes at 37 °C.



Wash the plate five times. Add HRP-Conjugate and incubate for 30 minutes at 37 °C.



Wash the plate five times. Add Chromogen solution A and B. Incubate for 15 minutes at 37 °C for color development and add stop solution.



Read the OD value within 15 minutes.



Plot and Calculate.

CALCULATION

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration for each standard on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Rabbit CRP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor, for example 5 in this case.

ASSAY RANGE : 75 µg/L - 1200 µg/L.

PACKAGE SIZE : 96Tests.

SENSITIVITY : <5 µg/L.

Linearity: To assess linearity of the assay, samples containing and/or spiked with high concentrations of Rabbit CRP were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.

VALIDITY & STORAGE: As per CoA (at 2-8°C).