Rat C-Reactive Protein (CRP) ELISA Kit (Urine & Cell Culture Samples)

Introduction

C-Reactive Protein (CRP) is a liver protein composed of five identical nonglycosylated subunits, with a total molecular weight of 105 kDa. CRP has a variety of powerful effects related to immunology, inflammation, and coagulation. As a marker of low-level inflammation, CRP appears to predict future cardiovascular disease events among apparently healthy individuals. High plasma concentration of CRP was associated with increased risk of stroke, myocardial infarction, and peripheral vascular disease (1, 2, 3). CRP has also been associated with increased risks of fatal coronary events among high-risk male smokers and incident coronary disease among the elderly (4, 5). Studies have established the prognostic usefulness of CRP in the setting of angina (6). Originally used as a marker of acute inflammation, CRP has become a leading candidate as the measure of choice for estimating the inflammatory component of cardiovascular disease risk.

Principal of the Assay

The Rat C-Reactive Protein ELISA kit is designed for detection of rat CRP in urine and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures CRP in 3.5 hours. A polyclonal antibody specific for rat CRP has been pre-coated onto a microplate. CRP in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for CRP, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **CRP Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against CRP.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **CRP Standard:** Rat CRP in a buffered protein base (100 ng, lyophilized).
- **Biotinylated CRP Antibody (70x):** A 70-fold biotinylated polyclonal antibody against rat CRP (120 µl).
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store kit at 2-8^{0}C or -20^{0}C upon arrival up to the expiration date.
  Opened EIA Diluent may be stored for up to 1 month at 2-8^{0}C. Store reconstituted reagents at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel pipette)
- Deionized or distilled reagent grade water

Sample Collection and Storage

- **Urine:** Collect urine using sample pot. Centrifuge samples at 600 x g for 10 minutes and assay. Dilute samples 1:4 into EIA Diluent. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. The samples can be stored at -20^{0}C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8^{0}C.
- **CRP Standard:** Reconstitute the 100 ng of rat CRP Standard with 2.0 ml of EIA Diluent to generate a 50 ng/ml of solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the Standard solution (50 ng/ml) 1:2 with equal volume of EIA Diluent to produce 25, 12.5,

6.25, 3.13, 1.562 and 0.78 ng/ml. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20^{0}C.

Standard Point	Dilution	[CRP] (ng/ml)
P1	1 part Standard (50 ng/ml)	50.00
P2	1 part P1 + 1 part EIA Diluent	25.00
P3	1 part P2 + 1 part EIA Diluent	12.50
P4	1 part P3 + 1 part EIA Diluent	6.25
P5	1 part P4 + 1 part EIA Diluent	3.13
P6	1 part P5 + 1 part EIA Diluent	1.56
P7	1 part P6 + 1 part EIA Diluent	0.78
P8	EIA Diluent	0.00

- **Biotinylated CRP Antibody (70x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:70 with EIA Diluent. Any remaining solution should be frozen at -20^{0}C.
- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20^{0}C.

Assay Procedure

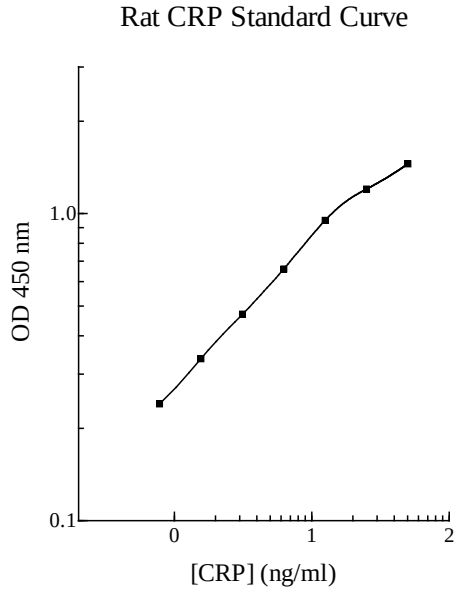
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 µl of Biotinylated CRP Antibody to each well and incubate for 1 hour.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 µl of Wash Buffer as above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that some unstable black particles may be generated at high optical densities to reduce the readings after stopping the reaction for about 10 minutes.

Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.
- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of CRP is typically less than 500 pg/ml.
- Intra-assay and inter-assay coefficients of variation were 5.0 % and 7.6% respectively.
- The kit can be used for rat plasma/serum samples. The suggested dilution is 1:20000.

Cross-Reactivity

Species	% Cross Reactivity
Beagle	<3
Bovine	None
Monkey	None
Mouse	< 10
Human	None
Swine	None
Rabbit	None

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Urine**	**Cell Culture**
1:4	101%	98%
1:8	100%	101%
1:16	105%	102%

Recovery

Standard Added Value	2 – 20 ng/ml
Recovery %	80-110 %
Average Recovery %	95 %

References

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(2) Ridker, P.M. *et al*. (1998) *Circulation* 98: 731
(3) Ridker, P.M. *et al*. (1998) *Circulation* 97: 425
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(5) Tracy, R.P. *et al.* (1997) *Arterioscler. Thromb. Vasc. Biol.* 17:1121
(6) Liuzzo, G. *et al.* (1994) *N. Engl. J. Med*. 331:417

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