



AssayMax Rat C-Reactive Protein (CRP) ELISA Kit (Plasma & Serum Samples)

Catalog # ERC1021-1

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 AssayMax Rat CRP ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of rat CRP in plasma, serum and cell culture supernatants. This assay employs a quantitative competitive enzyme immunoassay technique that measures rat CRP in less than 3 hours. A polyclonal antibody specific for rat CRP has been pre-coated onto a 96-well microplate with removable strips. CRP in standards and samples is competed by a biotinylated CRP sandwiched by the immobilized antibody and 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

- **Rat CRP Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against rat 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.

- **Rat CRP Standard:** Rat CRP in a buffered protein base (25 µg, lyophilized).
- **Biotinylated Rat CRP:** 1 vial, lyophilized.
- **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 unopened kit at 2 - 8⁰C up to expiration date.
Opened EIA Diluent may be stored for up to 1 month at 2-8⁰C. Store reconstituted reagents at -20⁰C 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

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:400 into EIA Diluent. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:400 into EIA Diluent. The undiluted samples can be stored at -20⁰C or below for up to 3 months. 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 2-8⁰C.
- **CRP Standard:** Reconstitute the 25 µg of rat CRP Standard with 1.25 ml of EIA Diluent to generate a 20 µg/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 (20 µg /ml) 1:4 with EIA Diluent to produce 5, 1.25, 0.313 and 0.078 µg/ml . EIA Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20⁰C.

Standard Point	Dilution	[CRP] ($\mu\text{g/ml}$)
P1	1 part Standard (20 $\mu\text{g/ml}$)	20.000
P2	1 part P1 + 3 part EIA Diluent	5.000
P3	1 part P2 + 3 part EIA Diluent	1.250
P4	1 part P3 + 3 part EIA Diluent	0.313
P5	1 part P4 + 3 part EIA Diluent	0.078
P6	EIA Diluent	0.000

- **Biotinylated Rat CRP (1x):** Dilute Biotinylated Rat CRP with 4 ml EIA Diluent to produce a working solution. Allow to sit for 10 minutes with gentle agitation prior to use. Any remaining solution should be frozen at -20°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°C .

Assay Procedure

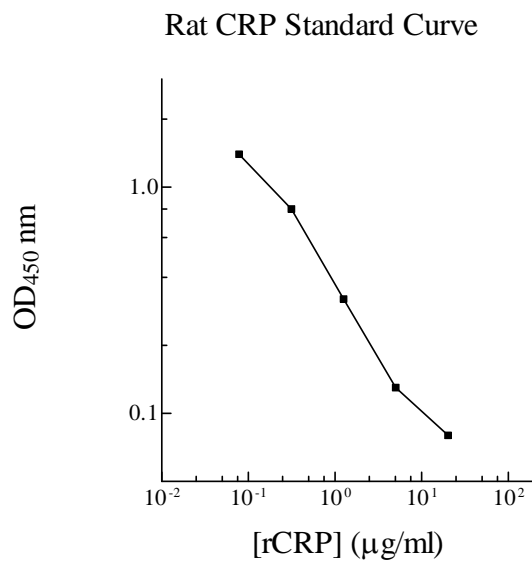
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature ($20-30^{\circ}\text{C}$).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 μl of standard or sample per well, and immediately add 25 μl of Biotinylated rat CRP to each well (on top of the Standard or sample) and mix gently. 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 completely remove liquid at each step.
- Add 50 μl of Streptavidin-Peroxidase Conjugate to each 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.
- Add 50 μl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap 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 after the reaction is stopped for about 10 minutes, some black particles may be generated at high concentration point, which will reduce the readings.

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 70 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.5 % and 7.6% respectively.

Cross-Reactivity

Species	% Cross Reactivity
Beagle	<1
Bovine	<1
Monkey	None
Mouse	< 10
Human	<5
Swine	<1

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:200	95%	98%
1:400	100%	101%
1:800	105%	106%

Recovery

Standard Added Value	0.5 – 8 µg /ml
Recovery %	84-110 %
Average Recovery %	97 %

Reference Value: Average rat plasma CRP concentration is 300 µg /ml

References

- (1) Ridker, P.M. *et al.* (1997) *N. Engl. J. Med.* 336: 973
- (2) Ridker, P.M. *et al.* (1998) *Circulation* 98: 731
- (3) Ridker, P.M. *et al.* (1998) *Circulation* 97: 425
- (4) Kuller, L.H. *et al.* (1996) *Am. J. Epidemiol.* 144:537
- (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

Version 1.1

Related Products

- EC1001-1 AssayMax Human CRP ELISA Kit
- ERC1001-1 AssayMax Rat CRP ELISA Kit (Urine & Cell Culture Supernatant)
- EMC1001-1 AssayMax Mouse CRP ELISA Kit