Human C-Reactive Protein ELISA Kit

Catalog No. IRAPKT044 Lot No. 11292

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.

Assay Principle

The Human C-Reactive Protein ELISA kit is designed for detection of human CRP in plasma, serum, saliva, milk, urine, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures CRP in less than 4 hours. A murine antibody specific for 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

- Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylatedantibody, and SP conjugate) as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.
- Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents.
- 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 murine 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:** *Human CRP in a buffered protein base (16 ng, lyophilized).*
- **Biotinylated CRP Antibody (50x):** *A 50-fold biotinylated polyclonal antibody against CRP (140 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, 2 bottles).*
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** *A 100-fold concentrate (80 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 and Condition

- Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Required Supplies

- 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 1:2000 with EIA Diluent. If necessary dilute samples within the range of 1:1000 to 1:4000. The undiluted samples can be stored at -20°C 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 1:2000 into EIA Diluent. If necessary dilute samples 1:1000 to 1:4000. The undiluted samples can be stored at -20°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°C or below. Avoid repeated freeze-thaw cycles.

Milk:

Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. Milk dilution is suggested at 1:30 into EIA Diluent; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Urine:

Collect urine using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Saliva:

Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preperation

Freshly dilute all reagents and bring all reagents to room temperature before use.

EIA Diluent Concentrate (10x): *If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8°C.*

Reagent Preperation Continued

CRP Standard: *Reconstitute the 16 ng of human CRP Standard with 1 ml of EIA Diluent to generate a 16 ng/ml of solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the Standard solution (16 ng/ml) 1:2 with equal volume of EIA Diluent to produce 8, 4, 2, 1, 0.5 and 0.25 ng/ml. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C.*

Biotinylated CRP Antibody (50x): *Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with EIA Diluent. Any remaining solution should be frozen at -20°C.*

Wash Buffer Concentrate (20x): *If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. 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*

Standard Point	Dilution	[CRP] (ng/ml)
P1	*1 part Standard (16 ng/ml)*	*16.00*
P2	*1 part P1 + 1 part EIA Diluent*	*8.00*
P3	*1 part P2 + 1 part EIA Diluent*	*4.00*
P4	*1 part P3 + 1 part EIA Diluent*	*2.00*
P5	*1 part P4 + 1 part EIA Diluent*	*1.00*
P6	*1 part P5 + 1 part EIA Diluent*	*0.50*
P7	*1 part P6 + 1 part EIA Diluent*	*0.25*
P8	*EIA Diluent*	*0.00*

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°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 manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
• Add 50 µl of Biotinylated CRP Antibody to each well and incubate for 30 minutes.
• Wash the microplate as described 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.

Assay Procedure Continued

• 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. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

• Calculate the mean value of the duplicate or 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.

Performance Characteristics

• The minimum detectable dose of CRP is typically ~ 0.25 ng/ml.
• Intra-assay and inter-assay coefficients of variation were 5.2 % and 7.1% respectively.

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	None
Monkey	5%
Mouse	10% (Suggest dilution 1:10 for Plasma)
Rat	None
Swine	None

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
1:1000	94%	93%
1:2000	99%	101%
1:4000	102%	104%