OriGene Technologies, Inc.
9620 Medical Center Dr., Suite 200, Rockville, MD 20850
Phone: 1.888.267.4436 Fax: 301-340-9254
Email: techsupport@origene.com Web: www.origene.com

Product Information

High Sensitivity C-Reactive Protein (CRP) ELISA kit

Catalog Number: EA100881
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The C-Reactive Protein Ultra Sensitive ELISA Kit is intended for the quantitative determination of C-reactive protein (CRP) in human serum or plasma.

Background

C-Reactive protein (CRP) is an alpha globulin with a molecular mass of approximately 110,000 to 140,000 daltons, and is composed of five identical subunits, which are noncovalently assembled as a cyclic pentamer. CRP is synthesized in the liver and is normally present as a trace constituent of serum or plasma at levels less than 0.3 mg/dl. CRP is one of the acute-phase proteins, the serum or plasma levels of which rise during general, nonspecific response to a wide variety of diseases. Although the detection of elevated levels of CRP in the serum is not specific for any particular disease, it is a useful indicator of inflammatory processes. Additionally, measurement of CRP by high-sensitivity CRP assays may add to the predictive value of other cardiac markers (myoglobin, creatine-kinase-MB, troponin I and T), which are used to assess the risk of cardiovascular and peripheral vascular disease. Inflammation in the arteries may play a role in heart disease and HS-CRP can determine heart disease risk in those with undetected heart disease and risk of complications for those who have already had a heart event.

Principle of the Test

The CRP ELISA kit is a solid phase direct sandwich method. The samples and conjugate reagent (anti-CRO biotin & HRP) are added to the wells coated with Streptavidin. CRP in the patient samples forms a sandwich between two specific antibodies to CRP. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the TMB substrate, the intensity of color is proportional to the concentration of CRP in the samples. A standard curve is prepared relating color intensity to the concentration of the CRP.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Streptavidin	12x8x1
2. CRP Standard: 6 vials (ready to use)	0.25 ml
3. CRP Enzyme Conjugate: 1 bottle (ready to use)	12 ml
4. TMB Substrate: 1 bottle (ready to use)	12 ml
5. Stop Solution: 1 bottle (ready to use)	12 ml

6. Sample Diluent: 2 bottles (2 x 25ml)	50 ml
7. 20X Wash Concentrate: 1 bottle	25 ml

Materials and Equipment Required but Not Provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

Reagent Preparation

1. **Wash Concentrate:** Prepare 1X Wash buffer by adding the Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

1. Place the desired number of coated strips into the holder
2. Dilute patient samples and controls 1:100 by adding 5 µl of samples to 495 µl of sample Diluent (STANDARDS ARE READY TO USE).
3. Dispense 10 µl of standard, diluted samples and controls into the appropriate wells
4. Add 100 µl of conjugate reagents to all wells. Tap the holder to remove air bubbles from the liquid and mix well.
5. Incubate for 60 minutes at room temperature (20-25°C).
6. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
7. Add 100 µl of TMB substrate to all wells.
8. Incubate for 15 minutes at room temperature.
9. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
10. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

Calculation of Results

The standard curve is constructed as follows:

1. Check CRP standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for the CRP standards (vertical axis) versus the CRP standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.
4. The obtained values of the patient samples and control sera should be multiplied by the dilution factor of 100 to obtain CRP results in mg/l.
5. Patient samples with CRP concentrations greater than 10 mg/l should be further diluted 10-fold after the initial 100-fold dilution (total dilution 1:1,000), and the final CRP values should be multiplied by 1,000 to obtain CRP results in mg/l.

Example of a Standard Curve

	OD 450	Conc. (mg/l)
Std 1	0.011	0
Std 2	0.168	0.005
Std 3	0.362	0.01
Std 4	1.000	0.025
Std 5	1.682	0.05
Std 6	2.399	0.1

Expected Values

It is recommended that each laboratory establish its own normal range based on the patient population. However, based on published literature healthy individuals are expected to have CRP values as follows: the CRP level in normal human serum ranges from 0.2 to 10 mg/l, where 90% of apparently healthy individuals have CRP levels <3 mg/l and only 1% have levels >10 mg/l.

Limitations of the Test

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

References

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Version 5, last updated October 28, 2022