



RABBIT C-REACTIVE PROTEIN ASSAY
FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
Cat. No. 921CRP01L-96

C-REACTIVE PROTEIN

C-reactive protein (CRP) is an acute-phase protein produced by the liver in conditions of inflammation, bacterial infection, and/or tissue trauma. Quantification of CRP is useful in determining inflammatory conditions difficult to diagnose, detect abnormalities in patients with chronic inflammatory conditions, and to monitor patients' response to treatment.

INTENDED USE

The HELICA Rabbit C-Reactive Protein Assay is a sandwich-based enzyme-linked immunoassay intended for the detection and quantification of rabbit CRP in rabbit serum, plasma, and fluids.

ASSAY PRINCIPLE

The HELICA Rabbit C-Reactive Protein Assay is species specific and provides a quantitative assessment of CRP levels in rabbit serum or plasma. The HELICA Rabbit C-Reactive Protein Assay is a sandwich-based enzyme-linked immunoassay in which the microtiter plates are coated with pneumococcal C-polysaccharide. After samples are prepared following the listed specimen collection procedure, they are applied to the antigen coated plate alongside the prepared standards. After incubation, the wells are decanted and washed to remove unreacted serum or plasma proteins, and a biotin-labeled anti-rabbit CRP antibody (conjugate) is added and reacts with the antigen-antigen complexes. After another incubation and wash step, avidin-peroxidase is added to bind to the biotin conjugate. Following another incubation period, the wells are decanted and washed to remove unreacted avidin. A hydrogen peroxide substrate with TMB as a chromogen is added to start color development. The intensity of the color is directly proportional to the amount of rabbit CRP in the sample. Therefore, the greater the intensity of the blue color, the higher the rabbit CRP concentration in the sample. The reaction is interrupted with a stop solution that turns the blue product yellow. The absorbance is read at a wavelength of 450nm on a spectrophotometer or plate reader.

REAGENTS PROVIDED

1 X Pouch	Antibody Coated Microwell Plate		96 wells (12 eight well strips) in a microwell holder coated with pneumococcal C-polysaccharide, <i>Ready-to-Use</i> .
1 X Vial	Rabbit CRP Standard (100X)	Green Cap Clear Vial	0.13mL of rabbit serum with elevated CRP concentration at 2.5µg/mL. Dilute standard stock 100X in Tris-T followed by four 2-fold serial dilutions to prepare standards at the following concentrations: 25.0, 12.5, 6.25, 3.13, and 1.56ng/mL.
1 X Vial	Biotin Anti-Rabbit CRP Conjugate (100X)	Amber Cap Amber Vial	0.13mL of 100X anti-rabbit CRP-IgG protein conjugated to biotin in buffer with preservative. Dilute conjugate in Tris-T to 1X prior to use.
1 X Vial	Avidin-Peroxidase Conjugate (100X)	Green Cap Amber Vial	0.13mL of 100X avidin conjugated to peroxidase in buffer with preservative. Dilute conjugate in Tris-T to 1X prior to use.
1 X Bottle	Substrate Reagent	Blue Cap	12mL stabilized tetramethylbenzidine (TMB), <i>Ready-to-Use</i> .
1 X Bottle	Stop Solution	Red Cap	12mL Acidic Solution, <i>Ready-to-Use</i> .
1 X Pouch	Washing Buffer		Tris with 0.05% Tween20, bring to 1L with distilled water and store refrigerated.

MATERIAL REQUIRED BUT NOT SUPPLIED

- Distilled or deionized water
- Wash bottle
- Dilution tubes
- Pipettor with tips: 2µL to 1000µL
- Adhesive cover for microplate
- Microplate reader with 450nm filter

PRECAUTIONS

1. Bring all reagents to room temperature (19°-27°C) before use.
2. Store reagents at 2°-8°C, and do not use beyond expiration date(s). Never freeze kit components.
3. Do not return unused reagents back into their original vials or bottles.
4. Do not interchange kit components between different lots of the same assay.
5. Adhere to all time and temperature conditions stated in the procedure.
6. Never pipette reagents or samples by mouth.
7. The Stop Solution contains acid. Do not allow to contact skin or eyes. If exposed, flush with water.
8. The standard serum and conjugate have not been screened for infectious agents. Since no testing can assure the absence of infectious agents, consider all materials, containers, and devices that are exposed to sample, standard, and conjugate to be contaminated with rabbit serum proteins. Wear protective gloves and safety glasses when using this kit.
9. Dispose of all materials, containers, and devices in the appropriate receptacle after use.
10. HRP-labeled conjugate and TMB substrates are photosensitive and are packaged in protective opaque bottles. Store in the dark and return to storage after use.

SPECIMEN COLLECTION AND PREPARATION

Specimens that are able to be used for this assay include: serum, plasma, urine, culture supernatant, tissue extracts, and synovial fluids.

Blood Samples

Blood samples should be collected using approved venipuncture techniques by qualified personnel. Allow sample to clot and separate serum by centrifugation. Transfer serum aseptically to a tightly closing sterile container. Store at 2-8°C. Alternatively, plasma extracted from blood drawn in heparin, EDTA, or ACD-containing tubes is acceptable. If testing is to be delayed longer than 5 days, freezing the sample at -20°C or below is recommended.

Upon specimen collection, dilute the sample 1:100 in Tris-Tween wash buffer (i.e. add 10µL of sample to 990µL of wash buffer).

Additionally, dilute the 1:100 sample 1:10 in Tris-Tween wash buffer (i.e. add 50µL sample to 450µL of wash buffer). **The final dilution for use in calculation is 1:1000.**

Specimens other than Blood (Serum or Plasma)

Samples other than blood (serum or plasma) should be prepared at higher concentrations. It is recommended to begin diluting the sample 1:2 in Tris-Tween wash buffer (i.e. add 100µL of sample to 200µL of wash buffer) and increasing the dilution factor accordingly. Dilution factor must be accounted for in the final calculation.

ASSAY PROCEDURE

Note: It is recommended that a multi-channel pipettor be utilized to perform the assay if more than 16 samples and standards (2 test strips) are run.

1. Bring all the reagents to room temperature before use. Reconstitute the Tris-Tween packet by washing out the contents with a gentle stream of distilled or deionized water into a 1-Liter container. Q.S. to 1 Liter with distilled or deionized water and store refrigerated when not in use.
2. Dilute 100X standard stock to 1X working concentration in Tris-Tween to prepare 25.0ng/mL standard. Prepare remaining standards by serially diluting standards 2-fold four times to yield 12.5, 6.25, 3.13, and 1.56ng/mL. Use Tris-Tween wash buffer for 0.0ng/mL standard. Consider the following dilution scheme as a guide:

Standard Concentration (ng/mL)	Tris-Tween Volume	Volume Transferred	Total Volume	Final Volume <small>*After serial dilution</small>
25.0	396µL	4µL	400µL	200µL
12.5	200µL	200µL	400µL	200µL
6.25	200µL	200µL	400µL	200µL
3.13	200µL	200µL	400µL	200µL
1.56	200µL	200µL	400µL	400µL

3. Using a new pipette tip for each, add 100µL of each standard and prepared sample into the appropriate wells. Samples and standards can be run in duplicate if desired. Incubate at room temperature for 30 minutes.
4. Decant the contents from the microwells into a discard basin. Wash the microwells by filling each with Tris-Tween wash buffer, then decanting the wash buffer into a discard basin. Repeat for a total of 5 washes.
5. Tap the microwells (face down) on a layer of absorbent towels to remove residual wash buffer.

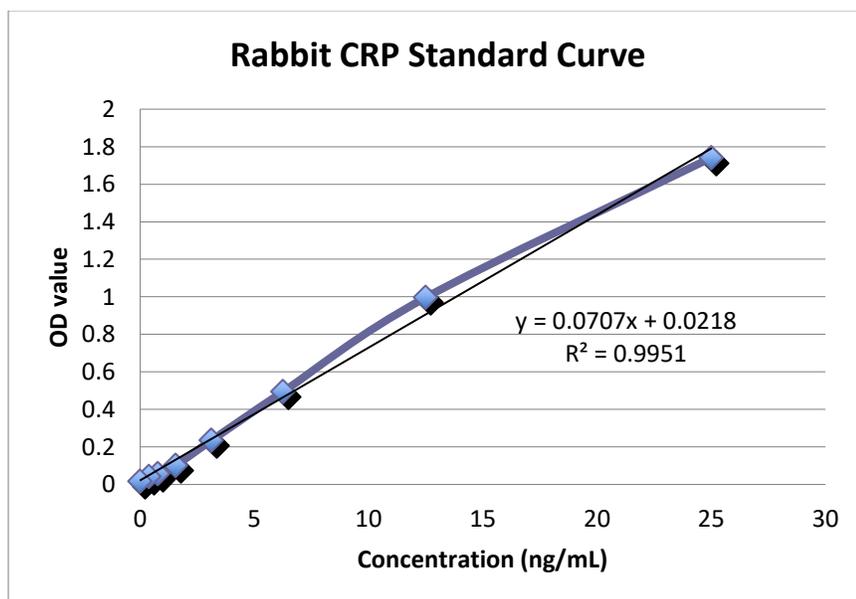
6. Determine the required volume of conjugate (1 mL/strip or 120 µL/well) to prepare. Dilute biotin labeled anti-rabbit CRP conjugate stock (100X) to working concentration (1X) with Tris-Tween wash buffer.
7. Add 100 µL of prepared biotin conjugate to each well. Incubate at room temperature for 30 minutes. Cover to avoid direct light.
8. Repeat steps 4 and 5.
9. Prepare the same volume of avidin-peroxidase conjugate as biotin labeled anti-rabbit CRP conjugate from Step 6. Dilute avidin-peroxidase conjugate stock (100X) to working concentration (1X) with Tris-Tween wash buffer.
10. Add 100 µL of prepared avidin-peroxidase conjugate to each well. Incubate at room temperature for 15 minutes. Cover to avoid direct light.
11. Decant the contents from the microwells into a discard basin. Wash the microwells by filling each with Tris-Tween wash buffer, then decanting the wash buffer into a discard basin. Repeat for a total of 10 washes.
12. Tap the microwells (face down) on a layer of absorbent towels to remove residual wash buffer.
13. Add 100 µL of Substrate Reagent to each well. Incubate at room temperature for 10 minutes. Cover to avoid direct light.
14. Add 100 µL of Stop Solution to each well in the same sequence and at the same pace as the Substrate Reagent was added.
15. Read the optical density (OD) of each microwell with a microtiter plate reader using a 450nm filter. Record the optical density (OD) of each microwell.

INTERPRETATION OF RESULTS

Construct a standard curve using the OD values against the concentration of the standards. Unknowns are measured by interpolation from the standard curve. Final concentration must be multiplied by the dilution factor at which samples were prepared to get the actual concentration (ng/mL). If a sample contains CRP at a greater concentration than the highest standard, it should be diluted appropriately in Tris-Tween and re-tested. The extra dilution step should be taken into account when expressing the final result.

ASSAY CHARACTERISTICS

Below is an example of a typical standard curve.



Intra-assay reproducibility

A typical example of the HELICA Rabbit CRP Assay used to test ten patient samples in four replicates yielded the following within assay variation.

	1:400 dilution	1:400 dilution	1:1,000 dilution	1:400 dilution		
Sample	OD	OD	OD	OD	Mean (µg/mL)	Std Dev
1	5.76	9.38	6.90	5.41	6.86	1.80
2	16.24	13.52	25.83	17.03	18.15	5.33
3	12.15	12.75	20.96	15.50	15.34	4.02
4	1.00	1.84	1.17	0.71	1.18	0.48
5	0.72	1.07	0.57	0.26	0.66	0.34
6	9.12	11.39	10.66	12.05	10.81	1.26
7	6.83	8.39	9.01	10.16	8.60	1.39
8	6.86	7.66	8.96	6.15	7.41	1.20
9	2.60	3.51	2.36	1.91	2.60	0.67
10	6.22	8.93	7.69	9.32	8.04	1.40

Inter-assay reproducibility

The HELICA Rabbit CRP Assay used to test ten patient samples with four different kit lots exhibited the following variability. Samples were diluted 1:1000, respectively.

Sample	OD	OD	OD	OD	Mean OD	Std Dev	%CV
1	0.614	0.636	0.57	0.607	0.607	0.027	4.5%
2	1.829	1.898	1.861	1.919	1.877	0.040	2.1%
3	1.488	1.554	1.483	1.657	1.546	0.081	5.2%
4	0.103	0.105	0.101	0.111	0.105	0.004	4.1%
5	0.061	0.073	0.06	0.066	0.065	0.006	9.1%
6	0.767	0.899	0.793	0.839	0.825	0.058	7.0%
7	0.652	0.759	0.723	0.767	0.725	0.052	7.2%
8	0.648	0.717	0.753	0.756	0.719	0.050	7.0%
9	0.186	0.183	0.189	0.198	0.189	0.006	3.4%
10	0.559	0.651	0.636	0.628	0.619	0.041	6.6%

Limitations

Lipemic sera may interfere with specific antibody reaction.

Limit of Detection

The lower and upper limit of quantitation is 1.56 and 25.0ng/mL, respectively.

Sensitivity

The HELICA Rabbit CRP Assay is designed to detect elevated levels of CRP. Experiments were performed to determine the sensitivity of the assay and may be useful in research applications where sensitivity parameters need to be defined. The detection limit calculated was: 0.39ng/mL.

Quality Control

It is recommended to routinely run at least two controls, each giving values at the top or bottom regions of the standard curve. An occasional prozone may be encountered in sera with high CRP values. In this situation, due to antigen excess, all the CRP available may not have reacted with the conjugate. Therefore, test at higher dilution (e.g. 1:2,000 and 1:4,000) to obtain more accurate results.

References

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