

Human CRP

HK358

Edition 02-12

ELISA KIT PRODUCT INFORMATION & MANUAL

Read carefully prior to starting procedures!
For use in laboratory research only
Not for clinical or diagnostic use



Note that this user protocol is not lot-specific and is representative for the current specifications of this product. Please consult the vial label and the Certificate of Analysis for information on specific lots. Also note that shipping conditions may differ from storage conditions.

For research use only. Not for use in or on humans or animals or for diagnostics. It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product.

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1. INTENDED USE

The human CRP ELISA kit is to be used for the *in vitro* quantitative determination of human CRP in serum and plasma. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.

The analysis should be performed by trained laboratory professionals.

2. INTRODUCTION

C-Reactive Protein (CRP) is an acute-phase protein, produced exclusively in the liver. Interleukin-6 is the mediator for the synthesis by the hepatocytes of CRP, a pentamer of approximately 120kDa. CRP is present in the serum of normal persons at concentrations ranging up to 5 µg/ml. The protein is produced by the fetus and the neonate and it does not pass the placental barrier, as such it can be used for the early detection of neonatal sepsis.

Because febrile phenomena, leukocyte count and erythrocyte sedimentation rate (ESR) are often misleading, investigators now prefer a quantitative CRP determination as a marker for acute inflammation and tissue necrosis. Within 6 hours of an acute inflammatory challenge the CRP level starts to rise.

Serum concentration of CRP increases significantly in cases of both infectious and non-infectious inflammation, of tissue damage and necrosis and in the presence of malignant tumors. CRP is present in the active stages of inflammatory disorders like rheumatoid arthritis, ankylosing spondylitis, Reiter's syndrome, psoriatic arthropathy, systemic lupus erythematosus, polyarteritis, ulcerative colitis and Crohn's disease.

Widespread malignant disease with carcinoma of the lung, stomach, colon, breast, prostate and pancreas, Hodgkin's disease, non-Hodgkin's lymphoma and lymphosarcoma will give rise to high levels of CRP resulting from tissue damage by invading tumor cells. Therefore, CRP may be used to monitor malignancy.

The CRP-level increases dramatically following microbial infections, and this may be particularly helpful for the diagnosis and monitoring of bacterial septicemia in neonates and other immunocompromised patients at risk. In children, CRP is useful for differential diagnosis of bacterial and viral meningitis.

Because the biological half-life of this protein is only 24 hours, CRP accurately parallels the activity of the inflammatory process and the CRP concentration decreases much faster than ESR or any other acute phase parameter, which is particularly useful in monitoring appropriate treatment of bacterial diseases with antibiotics.

CRP measurements during the early and late post-transplant period of bone marrow and organ transplantations is particularly useful in the management of interfering infections in these immunosuppressed patients.

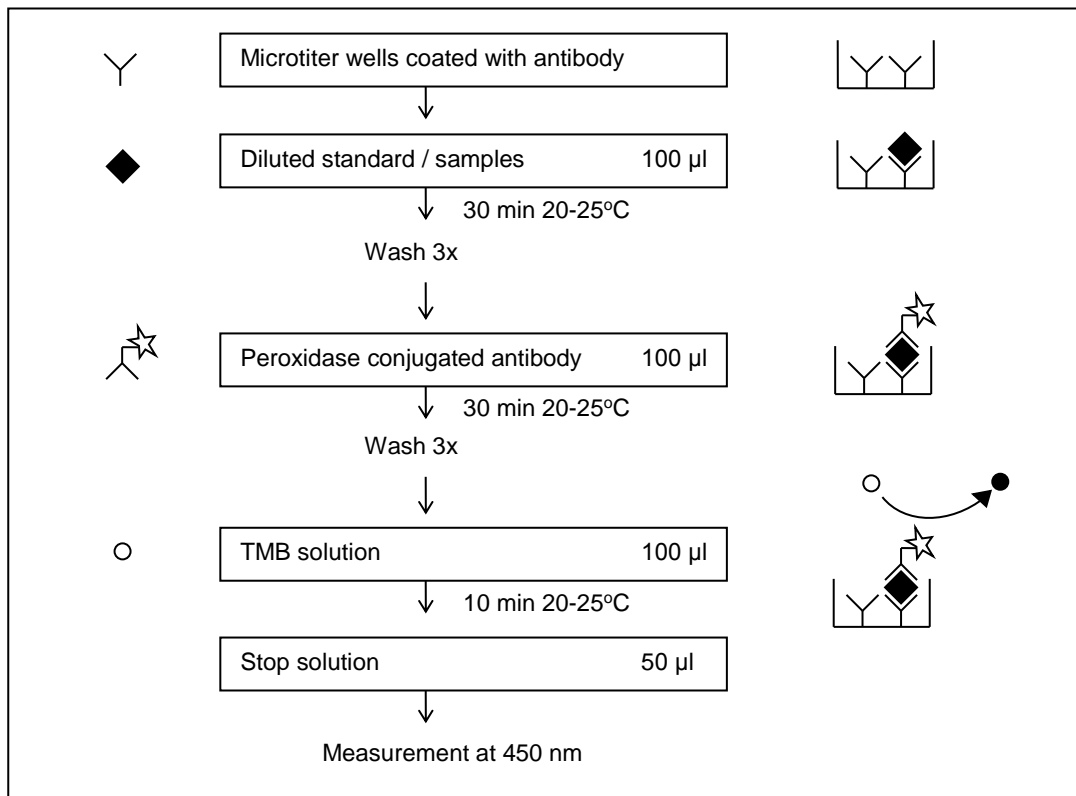
3. KIT FEATURES

- Working time of 1 hour and 10 minutes.
- Minimum concentration which can be measured is 5 ng/ml.
- Measurable concentration range of 5 to 100 ng/ml.
- Working volume of 100 µl/well.

Cross reactivity

Cross-reactivity for other species or proteins/peptides has not been tested.

4. PROTOCOL OVERVIEW



- The human CRP ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 1 hour and 10 minutes.
- The efficient format of a plate with twelve disposable 8-well strips allows free choice of batch size for the assay.
- Samples and standards are incubated in microtiter wells coated with antibodies recognizing human CRP.
- Peroxidase conjugate antibody will bind to the captured human CRP.
- Peroxidase conjugate antibody will react with the substrate, tetramethylbenzidine (TMB).
- The enzyme reaction is stopped by the addition of Sulfuric acid.
- The absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance (linear) versus the corresponding concentrations of the human CRP standards (log).
- The human CRP concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

5. KIT COMPONENTS AND STORAGE INSTRUCTIONS

Kit component	Quantity	Color dye
Wash buffer 20x	1 vial (50 ml)	
Sample Dilution buffer 5x	1 vial (40 ml)	Green
Standard A 0 µg/ml	1 vial (0.2 ml)	
Standard B 0.5 µg/ml	1 vial (0.2 ml)	
Standard C 2.5 µg/ml	1 vial (0.2 ml)	
Standard D 5 µg/ml	1 vial (0.2 ml)	
Standard E 10 µg/ml	1 vial (0.2 ml)	
Conjugate	1 vial (12 ml)	Red
TMB	1 vial (15 ml)	
Stop solution	1 vial (12 ml)	
12 Microtiter strips, pre-coated	1 plate	
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Table 1

- Upon receipt, store individual components at 2 - 8°C. Do not freeze.
- Do not use components beyond the expiration date printed on the kit label.
- Upon receipt, foil pouch around the plate should be vacuum-sealed and unpunctured. Any irregularities to aforementioned conditions may influence plate performance in the assay.
- Return unused strips immediately to the foil pouch containing the desiccant pack. Quality guaranteed for 1 month if stored at 2 - 8°C.

Materials requires but not provided

- Calibrated micropipettes and disposable tips.
- Distilled or de-ionized water.
- Plate washer: automatic or manual.
Additional wash buffer can be ordered separately. Please contact your local distributor.
- Polypropylene tubes.
- Calibrated ELISA plate reader capable of measuring absorbance at 450 nm.
- Adhesive covers can be ordered separately. Please contact your local distributor.

6. WARNINGS AND PRECAUTIONS

- For research use only, not for diagnostic or therapeutic use.
- This kit should only be used by qualified laboratory staff.
- Do not mix reagents from different kits and lots. The reagents have been standardized as a unit for a given lot. Use only the reagents supplied by the manufacturer.
- The assay has been optimized for the indicated standard range.
- Do not ingest any of the kit components.
- Some kit components contain sodium azide as a preservative. In order to prevent the formation of potentially explosive metal azides in laboratory plumbing, flush drains thoroughly after disposal of these solutions.
- The TMB substrate is light sensitive, keep away from bright light. The solution should be colorless until use.
- The stop solution contains 0.5M sulfuric acid and can cause irritation or burns to respiratory system, skin and eyes. Direct contact with skin and eyes should be strictly avoided. If contact occurs, rinse immediately with plenty of water and seek medical advise.
- Incubation times, incubation temperature and pipetting volumes other than those specified may give erroneous results.
- Do not reuse microwells or pour reagents back into their bottles once dispensed.
- Handle all biological samples as potentially hazardous and capable of transmitting diseases.
- Hemolyzed, hyperlipemic, heat-treated or contaminated samples may give erroneous results.
- Use polypropylene tubes for preparation of standard and samples. Do not use polystyrene tubes or sample plates.
- Human blood components used in the preparation of the standard sera have been tested and found to be nonreactive for hepatitis B surface antigen and HIV I. Since no known method can ever offer complete assurance that products derived from human blood will not transmit hepatitis or other viral infections, it is recommended to handle these standard sera in the same way as potentially infectious material. Dispose patient samples and all materials used to perform this test as if they contain infectious agents.

7. SAMPLE PREPARATION

Collection and handling

Plasma

Collect blood using normal aseptic techniques. Blood samples should be kept on ice. If serum is used, separate serum from blood after clotting at room temperature within 1 hour by centrifugation (1500xg at 4°C for 15 min). Transfer the serum to a fresh polypropylene tube.

If plasma is used, separate plasma from blood within 20 minutes after blood sampling by centrifugation (1500xg at 4°C for 15 min). Transfer the plasma to a fresh polypropylene tube.

Serum

Allow freshly collected blood to clot by standing tubes vertically at room temperature for 60 min. Centrifuge the clotted blood (1500xg at 4°C for 15 min). Transfer the serum to a fresh polypropylene tube.

Storage

Store samples below -20°C, preferably at -70°C in polypropylene tubes. Storage at -20°C can affect recovery of human CRP. Use samples within 24 hours after thawing. Avoid multiple freeze-thaw cycles which may cause loss of human CRP activity and give erroneous results.

Do not use hemolyzed, hyperlipemic, heat-treated or contaminated samples.

Before performing the assay, samples should be brought to room temperature (18 – 25°C) and mixed gently. Prepare all samples (controls and test samples) prior to starting the assay procedure. Avoid foaming.

Dilution procedures**Serum or plasma samples**

The patient samples are diluted 1:1000 in two consecutive steps: pipette 10 µl of each patient sample into separate glass dilution tubes and add 990 µl of diluted Sample Dilution Buffer. Mix thoroughly. Add 450 µl of diluted Sample Dilution Buffer to 50 µl of these 100 x prediluted samples. Mix thoroughly.

Warning: do not store the diluted samples for more than 8 hours.

8. REAGENT PREPARATION

Allow all the reagents to equilibrate to room temperature (20 – 25°C) prior to use. Return to proper storage conditions immediately after use.

Wash buffer

Dilute 50 ml of concentrated Washing Solution to 1000 ml with distilled water. In case less volume is required, prepare the desired volume of wash buffer by diluting 1 part of the 20x wash buffer with 19 parts of distilled water.

Reconstituted solution can be stored at least 1 month, store at 2 – 8 °C.

At higher temperatures, the concentrated Washing Solution may appear cloudy without affecting its performance. Upon dilution, the solution will be clear.

Dilution buffer

Dilute 40 ml of the concentrated Sample Diluent to 200 ml with distilled water. In case less volume is required, prepare the desired volume of Sample Diluent by diluting 1 part of the 5x Sample Diluent with 4 parts of distilled water.

Reconstituted solution can be stored at least 3 months or as long as solution remains clear. Store at 2 – 8 °C.

Standard solution

The 10 x prediluted standard sera are diluted 1:100 as follows: pipette 10 µl of each calibrator into separate dilution tubes. Add 990 µl of diluted Sample Dilution Buffer and mix carefully.

Calibrated against the NIBSC 1st International Standard, 85/506. Contain 0,09 % NaN₃ and antimicrobial agents as preservatives.

Prepare each human CRP standard in polypropylene tubes by serial dilution of the standards with sample dilution buffer as shown in Table 2.

Tube	Volume sample dilution buffer	Volume standard	Final concentration (ng/ml)
1	990 µl	10 µl standard E	100 ng/ml
2	990 µl	10 µl standard D	50ng/ml
3	990 µl	10 µl standard C	25 ng/ml
4	990 µl	10 µl standard B	5 ng/ml
5	990 µl	10 µl standard A	0 ng/ml

Table 2

9. ELISA PROTOCOL

Bring all reagents to room temperature (20 - 25°C) before use.

1. The standard sera are diluted 1:100 as follows : pipette 10 µl of each calibrator into separate glass dilution tubes. Add 990 µl of diluted Specimen Dilution Buffer and mix carefully.
2. The patient samples are diluted 1:1000 in two consecutive steps: pipette 10 µl of each patient sample into separate glass dilution tubes and add 990 µl of diluted Specimen Dilution Buffer. Mix thoroughly. Add 450 µl of diluted Specimen Dilution Buffer to 50 µl of these 100 x prediluted samples. Mix thoroughly.
Warning: do not store the diluted samples for more than 8 hours.
3. Pipette 100 µl of the diluted calibrators and samples into each of a pair of adjacent wells.
4. Incubate the covered microtiterstrips for 30 ± 2 min at room temperature.
5. Wash the microtiterstrips three times with Washing Solution. This can either be performed with a suitable microtiterplate washer or by briskly shaking out the contents of the strips and immersing them in washing solution. During the third step, the washing solution is left in the strips for 2-3 min. Change washing solution for each cycle. Finally empty the microtiterstrips and remove excess fluid by blotting the inverted strips on adsorbent paper.
6. Add 100 µl of Conjugate Solution and incubate the covered microtiterstrips for 30 ± 2 min at room temperature.
7. Repeat the washing procedure as described in 5.
8. Add 100 µl of Chromogen Solution to each well.
9. Incubate for 10 ± 2 min at room temperature. Avoid light exposure during this step.
10. Add 50 µl of Stopping Solution to each well.
11. Determine the absorbance of each well at 450 nm within 30 min following the addition of acid.

10. INTERPRETATION OF RESULTS

- The average absorbance value of each calibrator is plotted against the corresponding CRP-value and the best calibration curve (e.g. log/linear) is constructed.
- Use the average absorbance of each patient sample obtained in the CRP-ELISA to determine the corresponding value by simple interpolation from the curve.
- Depending on the experience and/or availability of computer capability, other methods of data reduction may be used.
- If individual absorbance values differ by more than 15% from the corresponding mean value, the result is considered suspect and the sample should be retested.
- The mean absorbance of the zero standard should be less than 0.1.
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Samples that give a mean absorbance above the absorbance for the highest standard concentration are out of range of the assay. These samples should be retested at a higher dilution.

11. TECHNICAL HINTS

- User should be trained and familiar with ELISA assays and test procedure.
- If user is not familiar with the ELISA technique it is recommended that the user perform a pilot assay prior to evaluation of your samples. Perform the assay with a standard curve only following the instructions.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing wash buffer, fill with wash buffer as indicated for each cycle and do not allow wells to sit uncovered or dry for extended periods.
- Since exact conditions may vary from assay to assay, a standard curve must be established for every run. Samples should be referred to the standard curve prepared on the same plate.
- Do not mix reagents from different batches, or other reagents and strips. Remainders should not be mixed with contents of freshly opened vials.
- To avoid cross-contaminations, change pipette tips between reagent additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Waste disposal should be performed according to your laboratory regulations.

Technical support

Do not hesitate to contact our technical support team at support@hycultbiotech.com for inquiries and technical support regarding the human CRP ELISA.

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12. QUALITY CONTROL

The Certificate of Analysis included in this kit is lot specific and is to be used to verify results obtained by your laboratory. The absorption values provided on the Certificate of Analysis are to be used as a guideline only. The results obtained by your laboratory may differ.

This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

For optimal performance of this kit, it is advised to work according to good laboratory practice.

13. PERFORMANCE CHARACTERISTICS

Example of typical O.D. values:

Standards (ng/ml)	O.D. value
100	2.018
50	1.301
25	0.821
5	0.240
0	0.019

All individuals have small amounts of CRP in their blood. The upper limit of the normal range is situated between 5 and 8 µg/ml.

The minimal detectable concentration is < 1 µg/ml.

Precision

Intra Assay (n=10)	Level 1	Level 2
Mean (µg/ml)	5.2	48.3
SD (µg/ml)	0.27	3.3
%CV	5.12	6.84

Inter Assay (n=7)	Level 1	Level 2	Level 3
Mean (µg/ml)	4.3	31.0	67.2
SD (µg/ml)	0.6	3.6	8.5
%CV	14.3	11.6	12.7

14. TROUBLESHOOTING

Warranty claims and complaints in respect of deficiencies must be logged before expiry date of the product. A written complaint containing lot number of the product and experimental data shall be sent to support@hycultbiotech.com.

Suggestions summarized below in Table 3 can be used as guideline in case of unexpected assay results.

Low absorbance	High absorbance	Poor duplicates	All wells positive	All wells negative	Possible cause
•	•		•	•	Kit materials or reagents are contaminated or expired
•					Incorrect reagents used
•		•	•		Lyophilized reagents are not properly reconstituted
•	•	•	•	•	Incorrect dilutions or pipetting errors
•		•			Improper plastics used for preparation of standard and/or samples
•	•				Improper incubation times or temperature
		•			Especially in case of 37°C incubation: plates are not incubated uniformly
•					Assay performed before reagents had reached room temperature
•	•	•	•	•	Procedure not followed correctly
				•	Omission of a reagent or a step
		•			Poor mixing of samples
	•		•		Low purity of water
	•	•			Strips were kept dry for too long during/after washing
	•	•	•		Inefficient washing
	•	•			Cross-contamination from other samples or positive control
		•	•		TMB solution is not clear or colourless
•	•				Wrong filter in the microtiter reader
	•	•			Airbubbles
		•			Imprecise sealing of the plate after use
•					Wrong storage conditions
•					Lamp in microplate reader is not functioning optimally

Table 3

15. REFERENCES

1. Powell LJ; C - Reactive Protein - a Review Am. J. Med. Technol., 1979, 87: 138
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3. Helgeson N et al; C - Reactive Protein : Laboratory Medicine, Vol. 2 (Race G. J., Ed.), Harper & Row, Hagerstown, chapter 29 (1973).
4. Johnson HL et al; Applications of acute phase reactants in infectious diseases J. Microbiol. Immunol. Infect. 1999, 32: 73