Human C-Reactive Protein/CRP ELISA Kit

Cat. No.: H1509775 | Pack size: 96T | Storage: Store at 2-8°C

Product Information

Item	Description
Specifications & Purity	BioReagent,for enzyme immunoassay(ELISA)
Application	ELISA
Sample type	body fluids, cell culture supernatants, Plasma, Serum, tissue homogenates
Detection Range	0.78 ng/mL~50 ng/mL
Method Of Detection	Sandwich ELISA
Detected Species Object	Human
Specificity	Detecting Human C-Reactive Protein/CRP in samples, with no significant cross-reactivity to other homologs.
Detection instrument	Microplate reader
Detection wavelength	450nm
Storage Conditions	Store at 2-8°C
Shipped In	Wet ice
Stability And Storage	Each component has a shelf life of 6 months under corresponding storage conditions.

Product Description

This ELISA kit adopts the double-antibody sandwich ELISA method to detect the concentration of human CRP in samples. The human CRP capture antibody is pre-coated on the microplate. When samples or standards are added, human CRP binds to the capture antibody, while other unbound free components are removed by washing. Subsequently, biotin-labeled anti-human CRP antibody is added, which binds to human CRP to form a sandwich immune complex; residual free components are then removed by washing. Next, enzyme conjugate is added, and biotin specifically binds to the enzyme conjugate. Thereby, HRP on the enzyme conjugate is linked to the sandwich immune complex, with unbound components removed by another washing step. Finally, chromogenic substrate is added. If human CRP is present in the sample, the bound HRP catalyzes the oxidation of the colorless substrate to produce a blue product. After adding stop solution, the final reaction solution turns yellow. The absorbance value is measured at 450 nm using a microplate reader. The concentration of human CRP is positively correlated with the OD_{450} value. A standard curve is plotted based on standard samples, and the human CRP concentration in unknown samples can be calculated by matching their OD_{450} values against the standard curve.

Background Introduction

C-reactive protein (CRP) is an acute-phase protein whose plasma level rises sharply upon infection or tissue injury in the body. CRP can activate the complement system and enhance the phagocytosis of phagocytes, eliminating invasive pathogenic microorganisms as well as damaged, necrotic and apoptotic tissue cells, thus playing a vital protective role in the innate immune response.

CRP has high affinity for phosphatidylcholine residues and can bind to multiple endogenous and exogenous ligands to activate the classical complement pathway. Meanwhile, it limits the progression and intensity of inflammatory reactions in the late stage of complement activation. In addition, CRP exhibits opsonization and agglutination activities similar to IgG and complement, enhancing the phagocytic capacity of macrophages against various bacteria and foreign substances, and reducing abnormal immune responses caused by exposure to exogenous antigens.

Contents & Storage

Item No.	Appearance	Components	96T	Storage
H1509775A	—	Pre-coated Assay Plate	96 wells	2-8°C.
H1509775B	Liquid	Sample Diluent	30 mL	2-8°C.
H1509775C	Solid	Recombinant human CRP standard (lyophilized)	2 vials (50 ng/vial)	2-8°C.

Item No.	Appearance	Components	96T	Storage
H1509775D	Liquid	Biotin-labeled human CRP antibody	130 µL (Dilution ratio: 1:100)	2-8°C.
H1509775E	Liquid	Antibody Diluent	12 mL	2-8°C.
H1509775F	Liquid	Enzyme Conjugate (HRP- labeled Streptavidin)	130 µL (Dilution ratio: 1:100)	2-8°C.
H1509775G	Liquid	Enzyme Conjugate Diluent	12 mL	2-8°C.
H1509775H	Liquid	Concentrated Washing Buffer (25×)	30 mL	2-8°C.
H1509775I	Liquid	TMB Substrate	10 mL	2-8°C.
H1509775J	Liquid	Stop Solution	10 mL	2-8°C.
H1509775K	—	Plate Sealer	4 sheets	2-8°C.

Operating Procedures

1. Sample Preparation

(1) Select the corresponding pretreatment method according to sample type:

A. Cell Culture Supernatant: Centrifuge the supernatant at 100~500×g for 5 min to remove suspended particles.

B. Serum Sample: Allow whole blood to stand at room temperature for 0.5~2 h for natural coagulation and serum separation. Centrifuge at 4°C, 1000~2000×g for 10 min, collect the yellow supernatant. Avoid aspirating the precipitate. Place prepared serum on ice for standby; do not add preservatives or anticoagulants.

C. Plasma Sample: Anticoagulate whole blood with EDTA, mix well and place on ice. Centrifuge at 4°C, 1000~2000×g for 10 min, collect the yellow supernatant. Avoid aspirating the precipitate and store the prepared plasma on ice.

D. Tissue Homogenate / Body Fluid: Centrifuge to remove precipitates.

Notes:①If detection cannot be performed immediately, aliquot and store samples at -20°C after preparation; avoid repeated freeze-thaw cycles.

② Ensure samples are clear and transparent. Centrifuge to remove suspended matter before testing if necessary.

③ Do not use hemolytic, icteric, hyperlipidemic or contaminated samples to ensure accurate results.

(2) Sample Dilution

Refer to relevant literature to estimate the content of the target analyte in the sample, so as to determine the appropriate dilution ratio, ensuring the concentration of the target analyte in the diluted sample falls within the optimal detection range of the ELISA kit. Adopt different dilution protocols according to the concentration level of the target analyte:

① When the target analyte concentration ranges from 500 to 5000 ng/mL, dilute the sample at a ratio of 1:100, namely add 3 µL sample into 297 µL sample diluent;

② When the target analyte concentration ranges from 50 to 500 ng/mL, dilute the sample at a ratio of 1:10, namely add 25 µL sample into 225 µL sample diluent;

③ When the target analyte concentration ranges from 0.78 to 50 ng/mL, dilute the sample at a ratio of 1:2, namely add 100 µL sample into 100 µL sample diluent;

④ When the target analyte concentration is ≤ 0.78 ng/mL, the sample generally requires no dilution. The above protocols are for reference only. Please record the sample dilution method in detail during the experiment.

2. Pre-Assay Preparation

(1) Take the kit out of 4 refrigerator and equilibrate at room temperature for 20 min. If stored at -20, completely thaw all components first, then equilibrate for 20 min. Store remaining reagents at 4 or -20 promptly after use.

(2) Dilute the 25× concentrated wash buffer to 1× working wash buffer with double distilled water or deionized water.

(3) Preparation and Dilution of Recombinant Human CRP Standard(Prepare within 2 h before use, operate at room temperature 25~28 strictly)

① Prepare 50 ng/mL standard: Add 1 mL sample diluent into the standard vial, let stand for over 15 min, then invert and swirl repeatedly to fully dissolve.

② Perform serial two-fold dilution of the 50 ng/mL standard with sample diluent as follows (maximum concentration: 50 ng/mL; diluent serves as 0 ng/mL blank).

Tube No.	Diluent Volume (µL)	Reconstituted Standard Volume (µL)	Final Concentration (ng/mL)
A	0	1000	50
B	300	300 (from Tube A)	25
C	300	300 (from Tube B)	12.5
D	300	300 (from Tube C)	6.25
E	300	300 (from Tube D)	3.12

Tube No.	Diluent Volume (µL)	Reconstituted Standard Volume (µL)	Final Concentration (ng/mL)
F	300	300 (from Tube E)	1.56
G	300	300 (from Tube F)	0.78
H	300	0	0

Note: Discard any remaining standard after reconstitution and aliquoting.

(4) Preparation of Biotin-Labeled Anti-Human CRP Antibody Working Solution

① Calculate total volume (prepare an extra 100~200 µL to compensate for operational loss) based on 100 µL per well.

② Prepare working solution at the ratio of 1 µL biotin-labeled antibody to 99 µL antibody diluent, mix gently.

(5) Preparation of Enzyme Conjugate Working Solution (Prepare within 1 h before use)

① Calculate total volume (prepare an extra 100~200 µL for loss) based on 100 µL per well.

② Prepare working solution at the ratio of 1 µL enzyme conjugate to 99 µL enzyme conjugate diluent, mix gently.

3. Detection Protocol

(1) Determine the required number of microplate strips, place them into the plate frame. Seal unused strips in the original aluminum foil bag and store at 4°C or -20°C.

Notes:

① *It is recommended to run duplicate wells for standards and samples.*

② A standard curve must be generated for each independent assay.

(2) Add 100 µL of diluted samples and serially diluted standards into corresponding wells respectively.

Seal the plate with sealing film and incubate at 37°C for 90 min.

Notes: ①If the estimated sample concentration exceeds the upper limit of the standard curve, further dilute the sample before testing.

② Complete the loading process within 10 min to avoid result deviation.

(3) Discard the liquid in the microplate without washing; pat the plate dry on absorbent paper.

(4) Add 100 µL prepared biotin-labeled antibody working solution to each well, seal and incubate at 37°C for 60 min.

(5) Wash the plate 5 times: add 300 µL 1× wash buffer per well, keep 15~30 s between injection and aspiration. Pat dry on absorbent paper after washing.

Note: Sufficient washing is critical; inadequate washing will cause significant experimental error.

(6) Add 100 µL diluted enzyme conjugate working solution per well, seal and incubate at 37°C for 30 min in the dark.

(7) Wash the plate 5 times following the same method as step (5).

(8) Add 100 µL TMB substrate per well, seal and react at 37°C for 10~25 min in the dark.

Notes:

① *Keep TMB away from oxidants and metals during storage and use.*

② Optimal color development time varies with laboratory conditions; obvious blue gradient can be observed in the first 3~4 standard wells when the reaction is sufficient.

(9) Add 100 µL stop solution per well, mix gently. Immediately measure OD_{450} with a microplate reader, set 540 nm or 570 nm as the correction wavelength to obtain corrected absorbance (OD_{450}-OD_{540} or OD_{450}-OD_{570}).

Note: Complete OD value reading within 10 min.

4. Data Analysis

(1) Establish the standard curve: Take standard concentration as the X-axis and OD value as the Y-axis, fit the curve with 4-Parameter Logistic (4-PL) regression using professional software. Calculate sample concentration according to the sample OD value against the standard curve.

Notes: ①The assay is valid only when the OD deviation of duplicate wells is within 20%; use the average value as the final reading.

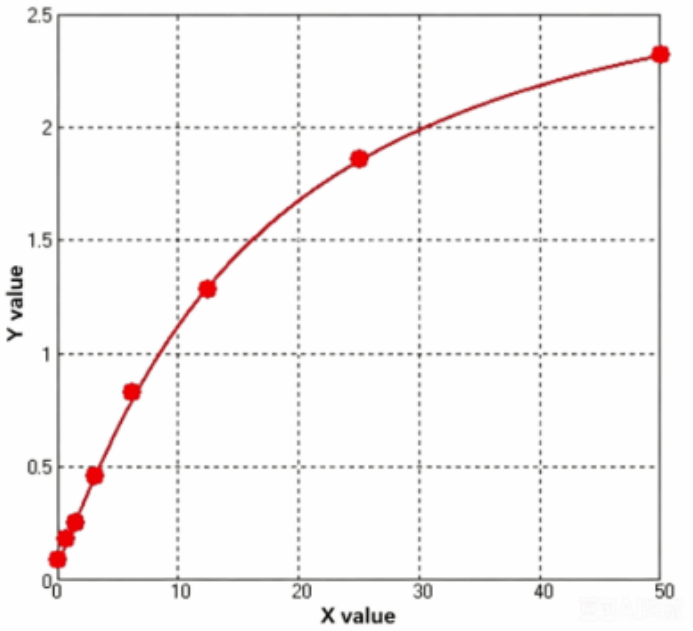
② If the sample OD value exceeds the upper limit of the standard curve, dilute the sample and re-test; multiply the result by the dilution factor when calculating the actual concentration.

(2) Typical Data

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Standard concentration (ng/ml)	O.D.
0	0.087
0.78	0.181
1.56	0.253
3.12	0.457
6.25	0.826
12.5	1.283
25	1.857

Standard concentration (ng/ml)	O.D.
50	2.316



(3) Precision

① Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

② Inter-Assay Precision (Precision between assays): Three samples of known concentration were tested in separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision	Intra-Assay Precision	Intra-Assay Precision	Inter-Assay Precision	Inter-Assay Precision	Inter-Assay Precision
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/ml)	64	73	105	84	49	193
Standard Deviation	5.1	2.5	5.4	2.6	1.8	8.9
CV%	7.9	3.4	5.1	3.1	3.7	4.6

(4) Recovery

The recovery of human C-Reactive Protein/CRP spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average Recovery (%)	Range (%)
Cell culture supernates (n=10)	113	90%-119%
Serum (n=10)	102	88%-110%
Heparin plasma (n=10)	97	90%-118%

(5) Linearity

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human C-Reactive Protein/CRP were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

Dilution Ratio	Index	Cell Culture Supernatants (n=10)	Serum (n=10)	Heparin Plasma (n=10)
1:4	Average of Expected (%)	92	92	104
1:4	Range (%)	83-120	83-97	93-119
1:8	Average of Expected (%)	101	97	101
1:8	Range (%)	86-106	96-102	89-118
1:16	Average of Expected (%)	100	90	100
1:16	Range (%)	82-108	88-94	92-111
1:32	Average of Expected (%)	93	98	103
1:32	Range (%)	90-104	86-99	96-115

Precautions

1. Crystals may form in concentrated washing buffer at low temperature. Warm in water bath to dissolve completely before use.

2. Do not mix components from different kit lots.

3. Avoid bubbles during pipetting. Ensure full mixing to avoid deviation.
4. Room temperature: strictly 25–28°C.
5. Wear lab coat and disposable gloves for personal safety.
6. For research use only, not for clinical diagnosis.

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