# ClinMax™ Human CRP ELISA Kit

Catalog Number：CRS-B016

Assay Tests: 96 tests

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

*Human CRP ELISA Kit User Guide*

**IMPORTANT: Please carefully read this user guide before performing your experiment.**

## Product information

This kit is specifically designed for the accurate quantitation of human CRP from cell culture supernates, serum and plasma.

The principle of this assay employs a quantitative sandwich enzyme immunoassay approach. Initially, a microplate is coated with a capture antibody. Then, samples and biotinylated capture antibody are added to the wells. After the removal of any unbound materials through washing, streptavidin-HRP (SA-HRP) conjugate is added to the wells. Streptavidin has a very high affinity for biotin, so it binds to the biotinylated capture antibody that is already bound to the target antigen. After washing, a substrate specific to HRP is added to the wells. HRP catalyzes a reaction that converts the substrate into a detectable signal, often a color change or luminescence, depending on the substrate used. This enzymatic reaction amplifies the signal, allowing for higher sensitivity in detecting the target analyte. The intensity of the signal is measured using a spectrophotometer.

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**NOTE:**

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Please do not use the kit after the expiration date indicated on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.

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## Contents

The kit contains sufficient reagents for 96 wells.

| Catalog | Contents | Amount |
|---|---|---|
| CRB016-C01 | Pre-coated Anti-CRP Antibody Microplate | 1 plate |
| CRB016-C02 | Human CRP Standard | 100 µL |
| CRB016-C03 | Biotin-Anti-CRP Antibody Con. Solution | 100 µL |
| CRB016-C04 | Biotin-Antibody Dilution Buffer | 8 mL |
| CRB016-C05 | Streptavidin-HRP Con. Solution | 500 µL |
| CRB016-C06 | Streptavidin-HRP Dilution Buffer | 15 mL |
| CRB016-C07 | 20× Washing Buffer | 50 mL |
| CRB016-C08 | 2×Sample Dilution Buffer | 15 mL×2 |
| CRB016-C09 | Substrate Solution | 12 mL |
| CRB016-C10 | Stop Solution | 6 mL |

## Storage

Keep the unopened kit stored at 2-8 °C. Avoid using the kit beyond its expiration date.
For opened kit, with the exception of the one contents listed in following table, others can be stored for up to 30 days at 2-8 °C.

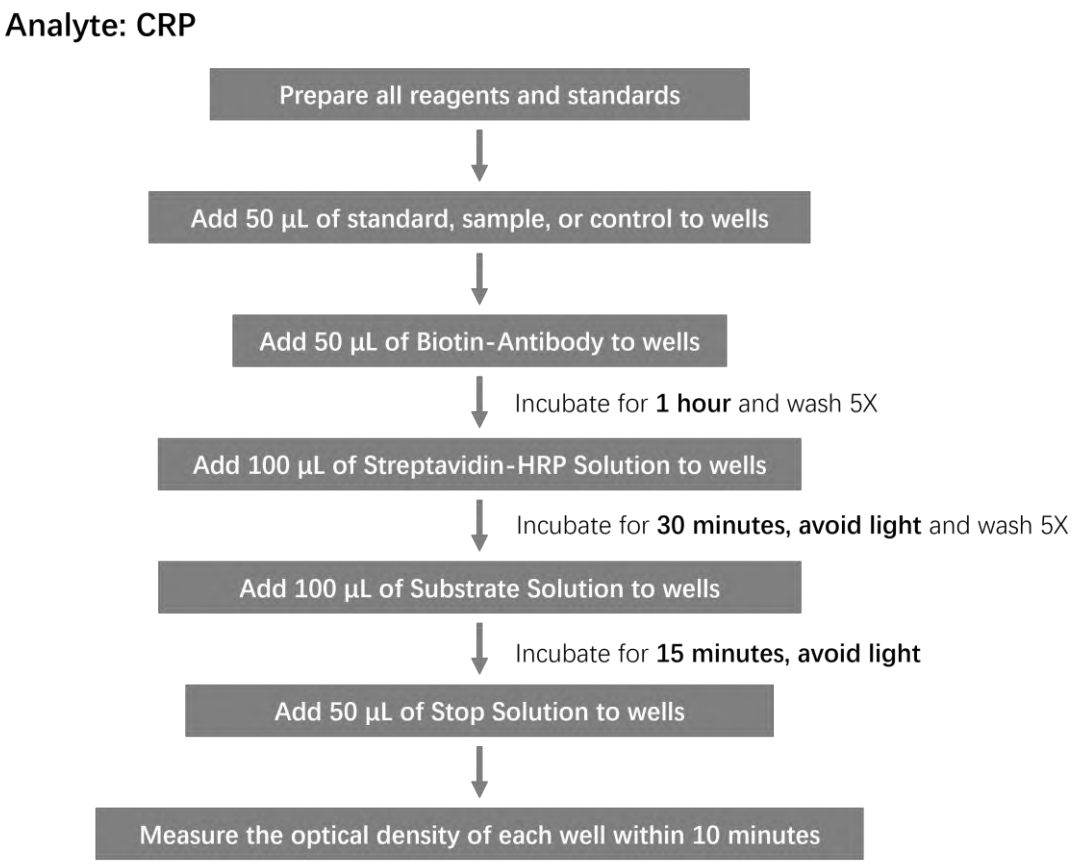
| Contents | Storage conditions |
|---|---|
| Pre-coated Anti-CRP Antibody Microplate | Return unused wells to the foil pouch, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8°C. |

***NOTE: Streptavidin-HRP Con. Solution and Substrate Solution should avoid light.***

## Required materials not supplied.

| | |
|---|---|
| **Instrument** | Microplate reader capable of measuring absorbance at 450 nm |
| **Reagents** | Deionized, ultrapure or distilled water |
| **Consumables** | 50 mL and 500 mL graduated cylinders |
| | Pipettes and pipette tips |
| | Tubes to prepare standard dilutions. |

## Workflow




*NOTE: Incubation temperature is 18 ℃-25 ℃*

## Prepare the working buffers and standard dilutions.

**IMPORTANT:** Bring all reagents to room temperature before use. If crystals have formed in buffer solution, place the buffer solution in an 37°C incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

**Prepare the working buffers.**

1. 1×Washing Buffer: Dilute 50 mL 20×Washing Buffer with deionized or distilled water to 1000 mL.
2. Biotin-Anti-CRP Antibody Solution: Add 60 µL of Biotin-Anti-CRP Antibody Con. Solution to 6 mL Biotin-Antibody Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
3. CRP Streptavidin-HRP Solution: Add 240 µL of CRP Streptavidin-HRP Con. Solution to 12 mL of Streptavidin-HRP Dilution Buffer, thoroughly mix. The solution was freshly prepared just before use.
4. Sample Dilution Buffer: Dilute 15 mL 2× Sample Dilution Buffer with deionized or distilled water to 30 mL.

**Prepare the standard serial dilutions.**

1. Label a tube "**Cm**". Add 10 µL of the reconstituted human CRP Standard and 990 µL of Sample Dilution Buffer to tube Cm, gently mix well.
2. Label 8 tubes, one for each standard point: Std.-1, Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7, Std.-8.
3. Add 20 µL of the liquid from **Cm** and 980 µL of Sample Dilution Buffer to tube Std.-1, thoroughly mix (Std.-1 =5000 pg/mL).
4. Prepare 1:1 serial dilutions for the standard curve as follows: Add 500 µL of Sample Dilution Buffer to each tube (Std.-2, Std.-3, Std.-4, Std.-5, Std.-6, Std.-7, Std.-8).
5. Transfer 500 µL of liquid from Std.-1 to the tube Std.-2, and thoroughly mix (Std.-2 = 2500 pg/mL).
6. Continue to transfer 500 µL of liquid from previous dilution tube to the next dilution tube until add liquid to tube Std.-8.
7. Sample Dilution Buffer serves as zero standard (blank).

**Preparation of sample (Suggested dilution ratio)**

Prepare S-1 by adding 10 µL of the liquid from sample (Serum, Plasma) to 240µL 1× Sample Dilution Buffer, and mix gently. Prepare S-2 by adding 10 µL of the S-1 to 190µL 1× Sample Dilution Buffer, and mix gently. S-2 is used for the assay.

## PROCEDURE OF ASSAY

1. Add 50 µL of CRP Standard, sample, or control to wells.
2. Add 50 µL Biotin-Anti-CRP Antibody Solution to each well, Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **1 hours.**
3. Aspirate each well and add 300 µL of 1×Washing Buffer to each well, gently tap the plate for **1 minute**. Remove any remaining Washing Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. Repeat the wash process four times for a total of five washes.
4. Add 100 µL of CRP Streptavidin-HRP Solution to each well. Seal the plate with microplate sealing film. Incubate at room temperature (18-25 °C) for **30 minutes, avoid light.**
5. Repeat step 3.
6. Add 100 µL of Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature (18-25 °C) for **15 minutes, avoid light**.
7. Add 50 µL of Stop Solution to each well. Tap the plate gently to ensure thorough mixing.

   ***Note:*** *the color in the wells should change from blue to yellow.*
8. Read the absorbance at 450nm and 630nm using Microplate reader within 10minutes.

   ***Note:*** *To reduce the background noise, subtract the readings at 630nm from the readings at 450nm.*

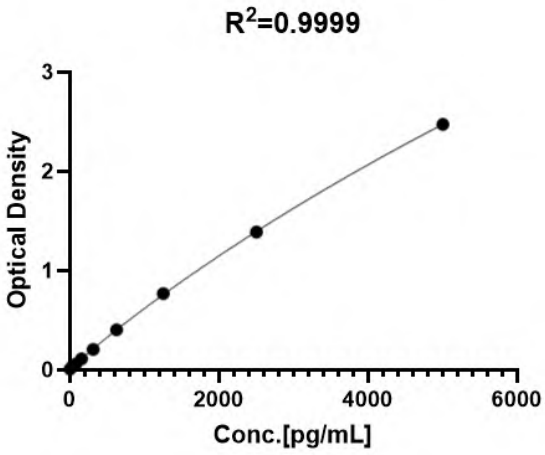
## CALCULATION OF RESULTS

1. Compute the average of the duplicated readings for every standard, control, and sample. Then, subtract the average optical density (O.D.) of the zero standard(blank).
2. Establish a standard curve by processing the data using computer software capable of executing a four-parameter logistic (4-PL) curve fitting.
3. Normal range of Standard curve: $R^2 \geqslant 0.9900$.
4. If the OD value of the sample to be tested is higher than the highest standard, the sample shall be diluted with dilution buffer and assay repeated.

## Typical data

***Note:*** *For each experiment, a standard curve needs to be set for each microplate, and the specific OD value may vary depending on different laboratories, testers, or equipment. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.*

| CRP Standard (pg/mL) | $OD_{450nm-630nm}$ |
|---|---|
| 5000 | 2.477 |
| 2500 | 1.390 |
| 1250 | 0.771 |
| 625 | 0.406 |
| 312.5 | 0.209 |
| 156.25 | 0.114 |
| 78.125 | 0.061 |
| 39.0625 | 0.041 |



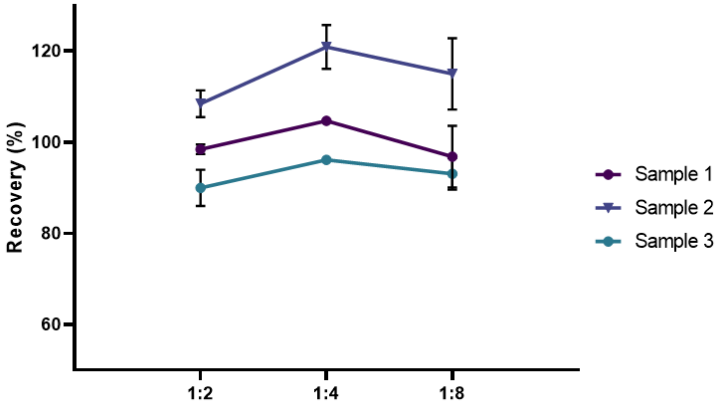
## PERFORMANCE CHARACTERISTICS

### 1. Sensitivity

The minimum detectable concentration (MDC) of CRP is typically less than 30 pg/mL. The MDC was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

### 2. Linearity

Three samples (Serum) spiked with high concentrations of CRP were serially diluted with dilution buffer to produce samples with values within the dynamic range of the assay and then assayed. The average recovery of CRP for serum samples is 98.56%.




### 3. Intra-Assay Precision

Ten replicates of each of 3 samples containing different CRP concentrations were tested in one assay. Acceptable criteria: CV < 10%.

| Sample Concentration (pg/mL) | Mean (pg /mL) | SD | Numbers | CV |
|---|---|---|---|---|
| 5000 | 4361.63 | 191.69 | 10 | 4.39% |
| 3750 | 3163.58 | 135.98 | 10 | 4.30% |
| 2500 | 2046.35 | 97.3 | 10 | 4.76% |

### 4. Inter-Assay Precision

Three samples containing different concentrations of CRP were tested in

independent assays. Acceptable criteria: CV<15%.

| Sample Concentration (pg/mL) | Mean (pg/mL)) | SD | Numbers | CV |
|---|---|---|---|---|
| 5000 | 4913.187 | 207.9558 | 9 | 4.23% |
| 2500 | 2565.302 | 63.02008 | 9 | 2.46% |
| 1250 | 1310.165 | 16.2587 | 9 | 1.24% |

## 5. Recovery

Recombinant CRP was spiked into 3 human serum samples, and then analyzed. The average recovery of CRP for serum samples is 80.49%.

| Sample ID | Conc Measured (pg/mL) | Conc Added ( pg/mL ) | Conc Recovered ( pg/mL ) | Recovery |
|---|---|---|---|---|
| 1 | 3986.61 | 3750 | 3181.01 | 84.83% |
| | 2811.35 | 2500 | 2005.75 | 80.23% |
| | 1861.78 | 1250 | 1056.18 | 84.49% |
| | 895.11 | | | |
| | | | | |
| 2 | 4313.59 | 3750 | 3297.57 | 87.94% |
| | 2902.92 | 2500 | 1886.90 | 75.48% |
| | 2135.53 | 1250 | 1119.51 | 89.56% |
| | 1128.92 | | | |
| | | | | |
| 3 | 3199.66 | 3750 | 3020.67 | 80.55% |
| | 2104.58 | 2500 | 1925.59 | 77.02% |
| | 1181.95 | 1250 | 1002.96 | 80.24% |
| | 198.87 | | | |
| | | | | |
| 4 | 3156.21 | 3750 | 2663.25 | 71.02% |
| | 2309.03 | 2500 | 1816.08 | 72.64% |
| | 1515.97 | 1250 | 1023.01 | 81.84% |
| | 547.73 | | | |

| | | | | |
|---|---|---|---|---|
| | | | | |
| 5 | 3395.40 | 3750 | 3036.52 | 80.97% |
| | 2306.72 | 2500 | 1947.84 | 77.91% |
| | 1391.31 | 1250 | 1032.43 | 82.59% |
| | 398.76 | | | |

## TROUBLESHOOTING GUIDE

| Problem | Cause | Solution |
|---|---|---|
| **Poor standard curve** | * Inaccurate pipetting | * Check pipettes |
| **Large CV** | * Inaccurate pipetting<br>* Air bubbles in wells | * Check pipettes<br>* Remove bubbles in wells |
| **High background** | * Plate is insufficiently washed<br>* Contaminated wash buffer | * Review the manual for proper wash.<br>* Make fresh wash buffer |
| **Very low readings across the plate** | * Incorrect wavelengths<br>* Insufficient development time | * Check filters/reader<br>* Increase development time |
| **Samples are reading too high, but standard curve looks fine** | * Samples contain cytokine levels above assay range | * Dilute samples and run again |
| **Drift** | * Interrupted assay set-up<br>* Reagents not at room temperature | * Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay<br>* Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts |