



MOUSE C-REACTIVE PROTEIN ASSAY

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Cat. No. 971CRP01M-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 Mouse C-Reactive Protein Assay is a sandwich-based enzyme-linked immunoassay intended for the detection and quantification of mouse CRP in mouse serum.

ASSAY PRINCIPLE

The HELICA Mouse C-Reactive Protein Assay is species specific and provides a quantitative assessment of CRP levels in mouse serum or plasma. The HELICA Mouse 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 an enzyme labeled anti-mouse CRP antibody (conjugate) is added and reacts with the antigen-antigen complexes. Following another incubation period, the wells are decanted and washed to remove unreacted conjugate. 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 mouse CRP in the sample. Therefore, the greater the intensity of the blue color, the higher the mouse 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 | Mouse CRP Standard (100X) | Green Cap | 0.13mL of mouse 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 | Anti-Mouse CRP Conjugate (100X) | Amber Cap | 0.13mL of 100X anti-mouse CRP-IgG protein 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 mouse 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

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 1:20 in Tris-Tween wash buffer (i.e. add 20µL of sample to 380µL of wash buffer). The final dilution for use in calculation is 1:20.

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 *After serial dilution |
|--------------------------------|-------------------|--------------------|--------------|--|
| 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 |

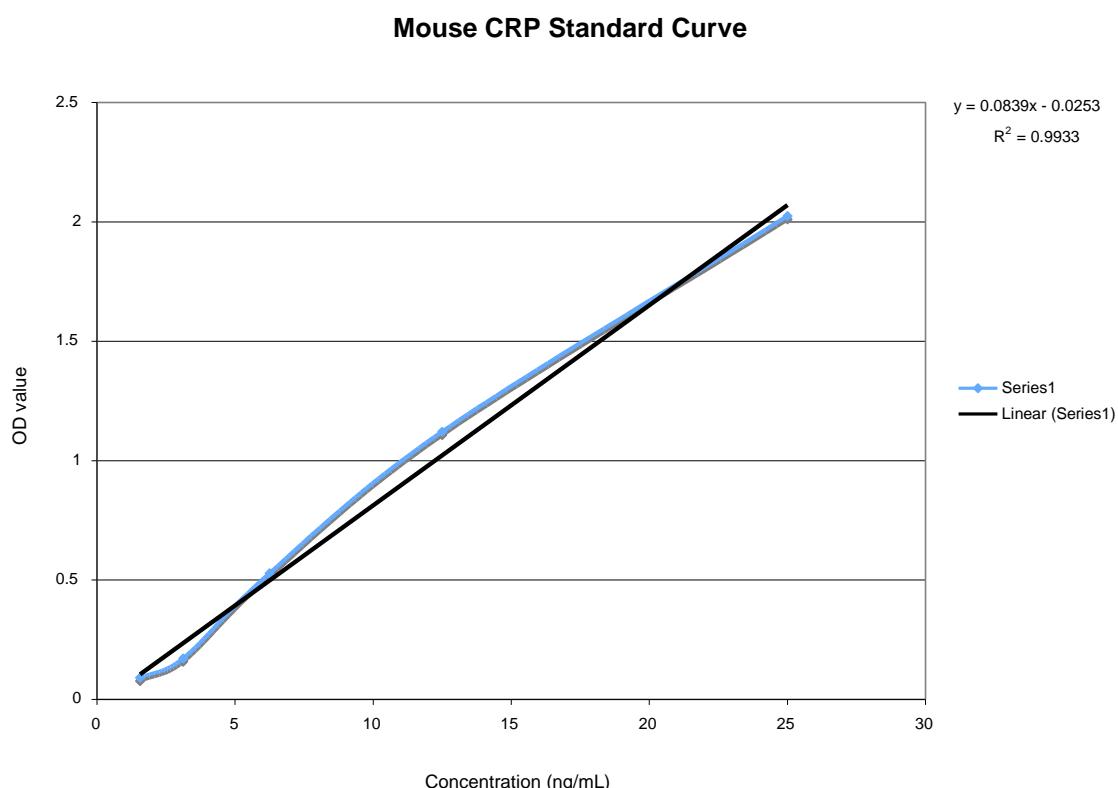
3. Using a new pipette tip for each, add 100µL of each standard and prepared sample into the appropriate wells. Samples and standards can be run in duplicate if desired. Incubate at room temperature for 1 hour.
4. Decant the contents from the microwells into a discard basin. Wash the microwells by filling 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 (1mL/strip or 120µL/well) to prepare. Dilute stock conjugate (100X) to working concentration (1X) with Tris-Tween wash buffer.
7. Add 100µL of conjugate to each well. Incubate at room temperature for 30 minutes. Cover to avoid direct light.
8. Repeat steps 4 and 5.
9. Add 100µL of Substrate Reagent to each well. Incubate at room temperature for 10 minutes. Cover to avoid direct light.
10. Add 100µL of Stop Solution to each well in the same sequence and at the same pace as the Substrate Reagent was added.
11. 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 Mouse CRP Assay run in 12 replicates yielded the following within assay variation.

| Standard (ng/mL) | %CV |
|------------------|-----|
| 25.0 | 3% |
| 12.5 | 3% |
| 6.25 | 6% |
| 3.13 | 4% |
| 1.56 | 6% |

Inter-assay reproducibility

The HELICA Mouse CRP Assay exhibited the following variability with two different kit lots performed 12 times.

| Standard (ng/mL) | %CV |
|------------------|-----|
| 25.0 | 6% |
| 12.5 | 7% |
| 6.25 | 6% |
| 3.13 | 5% |
| 1.56 | 8% |

Limitations

Lipemic sera may interfere with specific antibody reaction.

A study performed on 21 sera from healthy mice showed a range of 70-200ng/mL CRP in serum. Similar results are expected in plasma. Data on urine and other fluids are not available.

Limit of Detection

The lower and upper limit of quantitation is 1.25 and 12.5ng/mL, respectively.

Sensitivity

The HELICA Mouse CRP Assay is designed to detect elevated levels of CRP. The following data was produced to generate data on the sensitivity of the assay and may be useful in research applications where sensitivity parameters need to be defined (n=11).

| Mean OD | Standard Deviation | Detection Limit (ng/mL) |
|---------|--------------------|-------------------------|
| 0.087 | 0.008 | 1.5 |

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. 1:40 and 1:80) to obtain more accurate results.

Cross-Reactivity

| Species | % Cross-reactivity |
|---------|--------------------|
| Rat | 100% |

References

1. Ouchi N, Kihara S, Funahashi T, Nakamura T, Nishida M, Kumada M, Okamoto Y, Ohashi K, Nagaretani H, Kishida K, Nishizawa H, Maeda N, Kobayashi H, Hiraoka H, and Matsuzawa Y (2003). Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation.* 107(5):671-4.
2. Sasaki T, Motoyama S, Sato Y, Yoshino K, Matsumoto G, Minamiya Y, Saito H, Murata K, and Ogawa J (2013). C-reactive protein inhibits lymphangiogenesis and resultant lymph node metastasis of squamous cell carcinoma in mice. *Surgery.* 115(5):1087-92.
3. Teupser D, Weber O, Rao TN, Sass K, Thiery J, and Fehling HJ (2011). No Reduction of atherosclerosis in C-reactive protein (CRP)-deficient mice. *J Biol Chem.* 286(8):6272-9.