ab157712 –
C Reactive Protein
Mouse ELISA Kit

Instructions for Use

For the quantitative measurement of C Reactive Protein in mouse serum, plasma and other biological samples.

This product is for research use only and is not intended for diagnostic use.

Version 1 Last Updated 25 February 2013
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1. **BACKGROUND**

Abcam’s C Reactive Protein Mouse ELISA kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring C Reactive Protein in mouse biological samples.

In this assay the C Reactive Protein present in samples reacts with the anti-C Reactive Protein antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-C Reactive Protein antibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes with the previously bound C Reactive Protein. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3’,5,5’-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of C Reactive Protein in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of C Reactive Protein in the test sample. The quantity of C Reactive Protein in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.
2. ASSAY SUMMARY

Primary capture antibody

Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.

Sample

Add standard or sample to each well used. Incubate at room temperature.

HRP conjugated antibody

Aspirate and wash each well. Add prepared HRP labeled secondary detector antibody. Incubate at room temperature

Substrate  Colored product

Aspirate and wash each well. Add Chromogen Substrate Solution to each well. Immediately begin recording the color development
3. **PRECAUTIONS**
Please read these instructions carefully prior to beginning the assay.
All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. **STORAGE AND STABILITY**
Store kit at +2-8°C immediately upon receipt.
Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. **MATERIALS SUPPLIED**

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-Mouse C Reactive Protein ELISA Microplate</td>
<td>96 wells</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Mouse C Reactive Protein Calibrator (lyophilized)</td>
<td>1 vial</td>
<td>2-8°C</td>
</tr>
<tr>
<td>5X Diluent Concentrate</td>
<td>50 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>20X Wash Buffer Concentrate</td>
<td>50 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Enzyme-Antibody Conjugate</td>
<td>1 vial</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Chromogen Substrate Solution</td>
<td>12 mL</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>12 mL</td>
<td>2-8°C</td>
</tr>
</tbody>
</table>
6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Precision pipette (2 µL to 200 µL) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H₂O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer

7. LIMITATIONS

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.
- Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature.
- Do not mix or substitute reagents with those from other lots or sources.
8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
9. **REAGENT PREPARATION**

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

9.1 **5X Diluent Concentrate**

The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH₂O). The 1X working solution is stable for at least one week from the date of preparation and should be stored at 4-8°C.

9.2 **20X Wash Buffer Concentrate**

The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH₂O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals. The 1X working solution is stable for at least one week from the date of preparation and can be stored at room temperature (16-25°C) or at 4-8°C.

9.3 **Enzyme-Antibody Conjugate**

Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming. The working conjugate solution is stable for up to 1 hour when stored in the dark.

9.4 **Chromogen Substrate Solution**

Ready to use as supplied.

9.5 **Stop Solution**

Ready to use as supplied.
10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

10.1 Add 1.0 mL of distilled or de-ionized water to the Mouse C Reactive Protein Calibrator and mix gently until dissolved. The calibrator is now at a concentration of 265.83 ng/mL.

(Note: The reconstituted Mouse C Reactive Protein Calibrator should be aliquoted and stored frozen. Avoid multiple freeze-thaw cycles).

10.2 Label tubes #1-6:

10.3 To Tube#1, add 578 μL 1X Diluent + 60 μL of the reconstituted Mouse C3 Calibrator = Standard #1.

10.4 Add 250 μL 1X Diluent into Tubes#2-7.

10.5 Add 250 μL Standard #1 to tube #2 and mix thoroughly = Standard #2.

10.6 Transfer 250 μL from tube #2 to #3, mix thoroughly = Standard #3.

10.7 Using the table below as a guide, prepare further serial dilutions.

10.8 1X Diluent serves as the zero standard (0 ng/mL).
### Standard Dilution Preparation Table

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Volume to Dilute (µL)</th>
<th>Diluent (µL)</th>
<th>Total Volume (µL)</th>
<th>Starting Conc. (ng/mL)</th>
<th>Final Conc. (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>578</td>
<td>638</td>
<td>265.83</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>250</td>
<td>500</td>
<td>25</td>
<td>12.5</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>250</td>
<td>500</td>
<td>12.5</td>
<td>6.25</td>
</tr>
<tr>
<td>4</td>
<td>250</td>
<td>250</td>
<td>500</td>
<td>6.25</td>
<td>3.125</td>
</tr>
<tr>
<td>5</td>
<td>250</td>
<td>250</td>
<td>500</td>
<td>3.125</td>
<td>1.56</td>
</tr>
<tr>
<td>6</td>
<td>250</td>
<td>250</td>
<td>500</td>
<td>1.56</td>
<td>0.78</td>
</tr>
</tbody>
</table>

![Image of dilution process](image-url)
11. SAMPLE COLLECTION AND STORAGE

11.1 Serum – Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation.

11.2 Plasma – For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results.

Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

- Precautions
  For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

- Additives and Preservatives
  No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.
12. SAMPLE PREPARATION

General Sample information:
The assay for quantification of C Reactive Protein in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1/10 is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- To prepare a 1/10 dilution of sample, transfer 25 µL of sample to 225 µL of 1X diluent. This gives you a 1/10 dilution. Mix thoroughly.
13. PLATE PREPARATION

- The 96 well plate strips included with this kit is supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.
14. ASSAY PROCEDURE

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.

14.1 Pipette 100 μL of each standard, including zero control, in duplicate, into pre designated wells.
14.2 Pipette 100 μL of sample (in duplicate) into pre designated wells.
14.3 Incubate the micro titer plate at room temperature for ten (10 ± 2) minutes. Keep plate covered and level during incubation.
14.4 Following incubation, aspirate the contents of the wells.
14.5 Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.
14.6 Pipette 100 μL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for ten (10 ± 2) minutes. Keep plate covered in the dark and level during incubation.
14.7 Wash and blot the wells as described in 14.5.
14.8 Pipette 100 μL of TMB Substrate Solution into each well.
14.9 Incubate in the dark at room temperature for precisely five (5) minutes.
14.10 After five minutes, add 100 μL of Stop Solution to each well.
14.11 Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to manufacturer’s specifications.
15. **CALCULATIONS**

Average the duplicate standard reading for each standard, sample and control blank. Subtract the control blank from all mean readings. Plot the mean standard readings against their concentrations and draw the best smooth curve through these points to construct a standard curve. Most plate reader software or graphing software can plot these values and curve fit. A four parameter algorithm (4PL) usually provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4-parameter logistic). Extrapolate protein concentrations for unknown and control samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted in 1X Incubation Buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
16. **TYPICAL DATA**

**TYPICAL STANDARD CURVE** – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

![Typical Standard Curve](image)

<table>
<thead>
<tr>
<th>Conc. (ng/ml)</th>
<th>Mean O.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.78</td>
<td>0.144</td>
</tr>
<tr>
<td>1.56</td>
<td>0.263</td>
</tr>
<tr>
<td>3.125</td>
<td>0.479</td>
</tr>
<tr>
<td>6.25</td>
<td>0.882</td>
</tr>
<tr>
<td>12.5</td>
<td>1.588</td>
</tr>
<tr>
<td>25</td>
<td>2.414</td>
</tr>
</tbody>
</table>
17. **TYPICAL SAMPLE VALUES**

**SENSITIVITY –**

Calculated minimum detectable dose = 0.1691 ng/ml

**RECOVERY –**

Control Serum Recovery > 85%

**PRECISION –**

<table>
<thead>
<tr>
<th></th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-Assay</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Intra-Assay</td>
<td>&lt; 10</td>
</tr>
</tbody>
</table>
18. INTERFERENCES
These chemicals or biologicals will cause interferences in this assay causing compromised results or complete failure. Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

19. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Inaccurate pipetting</td>
<td>Check pipettes</td>
</tr>
<tr>
<td></td>
<td>Improper standards dilution</td>
<td>Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing</td>
</tr>
<tr>
<td>Low Signal</td>
<td>Incubation times too brief</td>
<td>Ensure sufficient incubation times; change to overnight standard/sample incubation</td>
</tr>
<tr>
<td></td>
<td>Inadequate reagent volumes or improper dilution</td>
<td>Check pipettes and ensure correct preparation</td>
</tr>
<tr>
<td>Large CV</td>
<td>Plate is insufficiently washed</td>
<td>Review manual for proper wash technique. If using a plate washer, check all ports for obstructions</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Prepare fresh wash buffer</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of the ELISA kit</td>
<td>Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.</td>
</tr>
</tbody>
</table>
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