ProGen Biologics

Serum/Plasma C3d Proceptor™ ELISA Kit Data Sheet (Product Code PG-C3d)

INTRODUCTION
Opsonization of circulating immune complexes (CICs) with C3 fragments is critical in maintaining a healthy immunologic balance. Factor I and co-factors including factor H covert C3b into iC3b and C3f. iC3b is cleaved into C3c and C3dg which is cleaved into C3d and C3g. C3d is a 35 kD protein and its concentration is reflective of the activity of the alternative complement pathway. The role for C3d-bound to antigen or IC is suggested in augmenting the humoral immune responses. C3d functions as a natural adjuvant to enhance the immune responses to antigens such as hen egg lysozyme, HIV gp120, influenza and measles virus hemagglutinin.

Proceptor™ (PG-C3d) ELISA kit measures the total C3d in plasma or serum sample. In conjunction with our C3d-CIC kit, these measurements can provide a good approach to assess the activity of the complement pathway and its participation in the disease process.

ASSAY PRINCIPLE
The C3d standards are coated in the wells in duplicate in row A1-H1 and A2-H2. To perform the test, an appropriate concentration of serum sample is incubated in the wells to allow binding of C3d. After washing away unbound proteins a detection antibody to C3d is added to all the wells and allowed to interact with the plate bound C3d. After washing, the wells are filled with species specific antibody-HRP enzyme reagent, species specific for the anti-C3d antibodies. After incubation, color is developed with TMB substrate. Intensity of the color developed is proportionate to the amount of C3d present in the sample.

REAGENTS
Microplate (300003d): Microplate (12X8 wells) coated with antibody to capture C3d. The C3d standards are coated from 1.56-200 ng/ml in doubling dilution, in rows A1-H1 and A2-H2.

Anti-C3d Primary Antibody (3000103d) (Mouse): One bottle containing 1.2 ml (10X) of anti-C3d antibody (purified globulin fraction). Dilute to 1X with wash buffer before use.


Color Development Reagent (1000901): One bottle containing 12 ml of chromogen TMB (tetramethylbenzidine).

Stop Solution (1000801): One bottle containing 7 ml of 0.25 N Sulfuric Acid.

Wash Buffer (1000601): Two bottles each contain 50 ml of 1X PBS/Tween-20. Prepare a working solution of wash buffer by adding 50 ml of 10X wash buffer to 450 ml of deionized water to make 500 ml of 1X wash buffer. The 1X wash buffer can be used to wash the plate or to dilute samples.

Block Buffer: One bottle of 1% BSA contain 20 ml buffer. Used for blocking plate and dilute to 0.5% for sample dilution using PBS.

SAMPLE PREPARATION
For C3d analysis by Proceptor™ ELISA suitable sample source are human plasma treated with heparin or serum. Sample is diluted 1:60 to 1:200 using 0.5% BSA/PBS buffer. We recommend diluting 15 µl in 1 ml for initial screening. For samples having higher concentration of C3d should be diluted appropriately and assayed again.

SAMPLE COLLECTION
Serum – Collect blood in serum separator tube and allow clotting for 30 minutes to 60 minutes before centrifugation at 1500 g., separate the serum and assay immediately or aliquot in small volumes for future use.

Plasma – Collect blood using EDTA and heparin as an anticoagulant. Centrifuge at 1500 g. within 30 minutes of collection. Assay immediately or store samples in aliquots.

SAMPLE STORAGE
Use serum or heparinized plasma samples immediately after collection or store sample preferably below -70 °C for future use. Avoid repeated freeze-thaw cycles. Sample stored at -20 °C can also be used.

Note: Hemolyzed samples and samples exposed to higher temperatures are not suitable for measurement of C3d. All reagents are optimized for this kit and are not interchangeable.

*ProGen’s proprietary receptors and their use are covered under US patent 7682793 B2. For research use only. Proceptor™ is a trade mark of ProGen Biologics, LLC
Note: The reagents should be brought to room temperature before using the test kit. Bring all solutions to room temperature (25°C).

ASSAY PROCEDURE
Any debris observed should be removed by centrifugation at 1500g for 10 minute at room temperature from the samples prior to sample dilution.

1. Dilute samples in 0.5% BSA/PBS. We recommend 1:60 to 1:200 dilution of the samples.
2. In the first step leave the first two rows A1-H1 and A2-H2 containing standards as it is without removing the plate sealer. These wells do not receive any solution in the first step.
3. Wash the wells in the rest of the plate (A3-H3 to A12-H12) with 200 µl of 1X wash buffer for two to three minutes.
4. Pipette 100 µl of sample per well. It is recommended to run the assay in duplicate wells.
5. Incubate the plate for ninety minutes at room temperature (20-25°C).
6. Wash 4-times times for a total of four washes with 300 µl of wash buffer using a squirt bottle or automated plate washer. Allow buffer to stay in the wells for 2 minutes between each wash.
7. Add 100 µl of anti-C3d antibody (diluted to 1X with 1X wash buffer) to each well and incubate the plate for sixty minutes at room temperature at room temperature (20-25°C).
8. Repeat wash as in step 6.
9. Add 100 µl of HRP-Conjugate and incubate the plate for another sixty minutes at room temperature at room temperature (20-25°C).
11. Add 100 µl of color development reagent and watch for appropriate development of color. It will take about 3 to 5 minute for color development, depending on the amount of C4d. During the development gently tap the plate occasionally to avoid trapping air bubbles in the substrate solution.
12. Stop the reaction by the adding 50 µl of stop reagent when desired optical density is reached.
13. Read the plate using a microplate reader at 450 nm.
14. Plot the standard values against the known concentration and using the linear equation calculate the sample values (y = a + bX).
15. Use the portion of the curve that is linear for calculating the values.

It is preferred to use plate shaker during incubations to obtain uniform binding.

CALCULATION OF RESULTS
Make a spreadsheet and enter the data. Average the duplicate readings for standards, controls and samples. Subtract the average zero standard optical density; if the function is available in the machine this can be done by the plate reader.

Create a standard curve by plotting the mean absorbance for each standard on the y-axis, against the concentration on the x-axis and draw the best fit curve through the points on the curve. Discard the values, which distort the curve or add undesired error. Calculate the slope and intercept use these values in a linear equation y = a+bx to calculate the sample values.

LIMITATION OF ASSAY
THE ASSAY IS FOR RESEARCH USE ONLY AND NOT FOR USE IN DIAGNOSTIC PROCEDURES.

• The samples should be prepared as described.
• It is important that the sample values fall within the linear part of the standard curve.
• The kit should be used within the expiration date.
• Variations in operator, pipetting technique, washing technique, incubation time, incubation temperature, kit age and presence of interfering agents in the serum and plasma samples may cause variation in binding.
Standard Curve for Plasma C3d

<table>
<thead>
<tr>
<th>Conc. Ng/ml</th>
<th>OD 450 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.56</td>
<td>0.013</td>
</tr>
<tr>
<td>3.13</td>
<td>0.029</td>
</tr>
<tr>
<td>6.25</td>
<td>0.065</td>
</tr>
<tr>
<td>12.50</td>
<td>0.153</td>
</tr>
<tr>
<td>25.00</td>
<td>0.373</td>
</tr>
<tr>
<td>50.00</td>
<td>0.885</td>
</tr>
<tr>
<td>100.00</td>
<td>1.646</td>
</tr>
<tr>
<td>200.00</td>
<td>2.691</td>
</tr>
</tbody>
</table>

Slope 0.0139
Intercept 0.0373

Use the linear portion of the curve for calculation.

The insert represents a typical standard curve; however some variation may occur due to the laboratory and user techniques.

Store Refrigerated
Batch No.:  
Expiry Date:
*ProGen’s proprietary receptors and their use are covered under US patent 7682793 B2. For research use only. Proceptor™ is a trade mark of ProGen Biologics, LLC*