**Fixed Complement 3d (C3d) Proceptor™ ELISA Kit Data Sheet (Product Code CC 003d)**

**INTRODUCTION**
The third complement component, C3 is a major complement factor due to its position in the classical, alternative, and lectin pathways and due to its relatively high concentration in the serum. The three pathways known to activate complement cascade lead to the formation of a convertase that cleaves C3 to C3a and C3b. The C3a is a known anaphylotoxin. The binding of C3b to immune complexes (ICs) facilitates the clearance of these proteins via binding through complement receptors present on the RBC. Opsonization of ICs with C3 fragments is critical in maintaining a healthy immunologic balance. Factor I and co-factors including factor H coverts 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 antigen or CIC is suggested in augmenting the humoral immune response. C3d is a molecular adjuvant of innate immunity that profoundly influences an acquired immune response. C3d stimulates antigen presentation by FDC and helps to maintain immunological B cell memory an essential step to generate disease-fighting antibodies. C3d has also been shown to enhance antibody titers.

Proceptor™ (CC-003d) ELISA kit is the only available assay, which measures the total C3d complement bound to the ICs. Assay provides a better indicator of immune status, since the assay measures the complement protein actively involved in immune physiological process.

**ASSAY PRINCIPLE**
The assay is based on unique capture of CICs by ProGen’s Proprietary Receptors. These purified receptors (Proceptor™) are pre-coated onto the microtiter plate. 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 containing CICs. The C3d within the CIC is then determined using C3d specific antibody and HRP conjugated secondary antibody. After washing the plate, a substrate solution (TMB) is added to all the wells for color development. Intensity of the color developed in proportion to the amount of C3d protein bound to the CICs.

**REAGENTS**

- **Receptor Microplate (3000103d):** Polystyrene microplate (12X8 wells) coated with receptors* specific for complexed immunoglobulins. Rows A1-H1 and A2-H2 are coated with varying concentrations of purified C3d protein standards. The standards are coated from (1.95 to 250 ng/ml) in doubling dilutions.

- **Anti-C3d Antibody (3000103d)(Rabbit):** One bottle containing 1.2 ml (10X) of anti-C3d antibody (purified globulin fraction) with stabilization solution and preservative. Dilute with 1X wash buffer before use.

- **HRP Conjugate (1000701d)(Goat):** One bottle containing 12 ml of secondary antibody against C3d antibody. Purified immunoglobulin fraction conjugated to Horseradish peroxidase (HRP).

- **Color Development Reagent (1000901):** One bottle containing 12 ml of stabilized 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 10X 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.

**SAMPLE PREPARATION**
Suitable samples for C3d analysis by Proceptor™ ELISA are Human plasma treated with heparin or human serum. EDTA treated plasma is not recommended for the assay. Serum or plasma is diluted 1:20 to 1:80 using 1X wash buffer. For samples having higher concentration of bound C3d should be diluted appropriately and assayed again so the measured optical density falls within the standard curve.

**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.

**CAUTION**
Hemolyzed samples and samples exposed to higher temperatures are not suitable for measurement of C3d. The use of serum is preferred for C3d measurement.

*ProGen’s proprietary receptors and their use are covered 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 with 1X wash buffer. We recommend 1:20 to 1:100 dilutions 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 to 25°C).
6. Wash 4-times 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 (20 to 25°C). Seal the plate with plastic sealers during each incubation period.
8. Repeat wash as in step 6.
9. Add 100 µl of HRP-Conjugate and incubate for sixty minutes at room temperature (20 to 25°C).
11. Add 100 µl of color development reagent and watch for appropriate development of color. It will take about 5 to 15 minute for color development, depending on the amount of C3d present on the CIC. During the development gently tap the plate occasionally to avoid trapping air bubbles in the substrate solution.
12. Stop the reaction by adding 50 µl of stop reagent, after the 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 the plate reader can do this.

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.

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Standard Curve for Fixed C3d

<table>
<thead>
<tr>
<th>Concentration (ng/ml)</th>
<th>OD Avg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.95</td>
<td>0.084</td>
</tr>
<tr>
<td>3.91</td>
<td>0.103</td>
</tr>
<tr>
<td>7.81</td>
<td>0.131</td>
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<tr>
<td>15.63</td>
<td>0.195</td>
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<tr>
<td>31.25</td>
<td>0.38</td>
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<tr>
<td>62.50</td>
<td>0.761</td>
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<tr>
<td>125.00</td>
<td>1.3825</td>
</tr>
<tr>
<td>250.00</td>
<td>2.3095</td>
</tr>
</tbody>
</table>

Slope: 0.009201
Intercept: 0.095442

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:

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