Fixed Complement C1q Proceptor™ ELISA Kit Data Sheet
(Product Code CC 001)

INTRODUCTION
C1q is a subcomponent of the first component of complement C1, which is a multimolecular complex comprising one molecule of C1q and two molecules each of the autoreactive proteases, C1r and C1s. This multimolecular complex triggers the classical pathway of complement. Opsonization of immune complexes (ICs) with C1q triggers the activation of classical pathway. Advances in the past several years have provided further insight into the multifunctional immune aspects of this molecule. Based on the data obtained from human diseases and animal models, it seems that the beneficial role of the early components such as C1q in opsonization and clearance of apoptotic cells and immune complexes supersedes the potentially damaging role mediated by downstream complement activation products. The deficiency of C1q complement protein is a known risk factor for the development of systemic lupus erythematosus (SLE). Anti-C1q antibodies (anti-C1q) have been shown to correlate positively with SLE. Anti-C1q autoantibodies are also reported to be present in various other rheumatic diseases.

Proceptor™ (CC-001) ELISA kit is the only available assay, which measures the total C1q complement protein bound to circulating immune complexes (CICs). This assay measures the complement protein, which is actively involved in immune physiological processes.

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. To perform the assay, appropriately diluted samples are incubated in the wells to allow binding of C1q containing CICs to the receptors. The C1q within the CIC is then measured using C1q specific antibody and HRP conjugated secondary antibody. After washing, the plate, a substrate solution (TMB) is added to the wells for color development.

Intensity of the color developed is proportionate to the amount of C1q protein bound to the CICs.

REAGENTS
Receptor Microplate (3000101): Polystyrene microtiter plate (12 X 8 wells) coated with receptors* specific for CICs. Rows A1-H1 and A2-H2 are coated with varying concentrations of purified C1q protein standards. The standards are coated from (6.25 to 200 ng/ml) in doubling dilutions.

Anti-C1q Antibodies (Goat) (3000101): One bottle containing 1.2 ml (10X) of anti-C1q antibody (purified globulin fraction) with stabilization solution and preservative. Dilute with 1X wash buffer before use.

HRP Conjugate (Donkey) (1000701): One bottle containing 12 ml of secondary antibody against C1q antibody. Purified immunoglobulin fraction conjugated to Horse radish peroxidase (HRP).

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

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

Wash Buffer (1000601): Two bottles each containing 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 and to dilute samples.

SAMPLE PREPARATION
Suitable samples for C1q analysis by Proceptor™ ELISA: Human plasma treated with heparin and human serum. EDTA treated plasma is not recommended for the assay. Serum or plasma is diluted 1:10 to 1:20 using 1X wash buffer. Samples with higher concentration of bound C1q 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 C1q.

Note: The reagents should be brought to room temperature before using the test kit. Bring all solutions to room temperature (20-25°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 1:10 to 1:20 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 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 diluted 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-C1q antibody (diluted to 1X with 1X wash buffer) to all wells and incubate the plate for sixty minutes at room temperature (20 to 25°C).
8. Repeat wash as in step 6.
9. Add 100 µl of anti-C1q-HRP-Conjugate to all wells and incubate for another 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 to develop, depending on the amount of C1q present on the CIC. During color development gently tap the plate occasionally to avoid trapping air bubbles in substrate solution.
12. Stop the reaction by addition of 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, 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.

*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*
Standard Curve for Fixed C1q

<table>
<thead>
<tr>
<th>Conc. ng/ml</th>
<th>O D 450 nm</th>
</tr>
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<tbody>
<tr>
<td>6.25</td>
<td>.207</td>
</tr>
<tr>
<td>12.5</td>
<td>.305</td>
</tr>
<tr>
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<td>.508</td>
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<tr>
<td>50</td>
<td>1.087</td>
</tr>
<tr>
<td>100</td>
<td>1.852</td>
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</tbody>
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Slope 0.01771
Intercept 0.11925

Use the linear portion of the curve for calculations.
The insert represents a typical standard curve; however some variation may occur due to the laboratory and user techniques.

Store Refrigerated
Expiry Date:
Batch No.:
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