Fixed Complement 4 (C4) Proceptor™ ELISA Kit Data Sheet (Product Code CC 004)

INTRODUCTION
The fourth complement component is a key protein of the classical pathway. C4 molecules is cleaved by C1 complex C4a and C4b. C4b has a very short-lived and has highly reactive binding site, which allows C4b to bind covalently to the nearest hydroxyl and amino groups, usually located on antigenic surfaces such as antibody coated viral particles and bacterial membranes. Opsonization of immune complexes (ICs) with C4 fragments is critical in maintaining a healthy immunologic balance and is a good indicator of classical pathway activation. The deficiency of C4 protein is linked to predisposition for the development of Systemic Lupus Erythematosus (SLE). Measurement of C4 bound to circulating immune complexes (CIC) is a good measure of disease activity in autoimmune disorders.

Proceptor™ (CC-004) ELISA kit is the only available assay, which measures the total C4 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 a 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 C4 containing CICs to the receptors. The C4 within the CIC is then measured using C4 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 is proportionate to the amount of C4 protein bound to CICs.

REAGENTS
Receptor Microplate (3000104): Polystyrene microplate (12X8 wells) coated with receptors specific for CICs. Rows A1-H1 and A2-H2 are coated with doubling concentration of purified C4 protein standards. The standards are coated from (1.56 to 200 ng/ml) in doubling dilutions.

Anti-C4 Antibodies (3000104) (Goat): One bottle containing 1.2 ml (10X) of anti-C4 antibody (purified globulin fraction) with stabilization solution and preservative. Dilute with 1X wash buffer before use.

HRP Conjugate (1000704) (Donkey): One bottle containing 12 ml of secondary antibody against C4 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 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 C4 analysis by Proceptor™ ELISA include human plasma treated with heparin and human serum. EDTA treated plasma is not recommended for the assay. Serum or plasma is diluted 1:20 to 1:60 with wash buffer. Samples with higher concentration of bound C4 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 C4. The use of serum is preferred for C4 measurement.

Note: The reagents should be brought to room temperature before using the test kit. Bring all solutions to room temperature (20-25°C).

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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 1:60 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 25ºC).
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-C4 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 to 25ºC).
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 to 25ºC).
11. Add 100 µl of color development reagent and watch for appropriate development of color. It will take about 5 to 10 minute for color development, depending on the amount of C4 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 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.

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 clear and 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 C4

<table>
<thead>
<tr>
<th>Conc. ng/ml</th>
<th>O.D. 450 nm</th>
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<tr>
<td>100</td>
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<tr>
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<tr>
<td>25</td>
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<td>3.12</td>
<td>0.1005</td>
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<td>0.0735</td>
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<tr>
<td>Slope</td>
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<tr>
<td>Intercept</td>
<td>0.039249</td>
</tr>
</tbody>
</table>

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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