Circulating Immune Complexes
IgG-CIC Proceptor™ ELISA Kit
Data Sheet (Product Code IC 001)

INTRODUCTION
Formation of antigen-antibody complexes also known as immune complexes (ICs) is a normal physiological process for the removal of foreign substances. However the patho-physiological events can lead to an excessive formation and poor clearance of IC that could lead to deposition of these ICs at various tissue sites. The deposition of ICs triggers the tissue damage at the deposited sites. Increased levels of ICs have been demonstrated in infectious and autoimmune disorders. The diseases i.e. SLE and Guillain-Barre syndrome is complication of serum sickness. The clinical disease coincides with the development of high levels of circulating IC and marked decrease in serum levels of C3, C4 and complement hemolytic activity.

A number of methods have been employed for the measurement of ICs from patient sera and plasma. Due to highly dynamic nature of circulating immune complexes (CICs), the existing methods have limitations in terms of measuring the IC formed in antigen or antibody excess. Proceptor™ (IC-001) CIC-IgG ELISA is based on a receptor interaction, which binds to the constant region of complexed immunoglobulin. The assays efficiently detect the IgG containing complexes. We also offer ELISA assays for IgM and IgA containing CIC.

ASSAY PRINCIPLE
The assay is based on unique capture of circulating immune complexes by ProGen’s Proprietary Receptors. These purified receptors are coated onto the microtiter plate. The samples at appropriate concentration are pipetted into the wells, which allow the CIC to bind receptors on solid phase. After washing away any unbound substances, the secondary antibody, a polyclonal antibody specific to IgG γ chain-HRP conjugated is added to each well of the mirotiter plate. The color is developed using substrate (TMB) solution. Intensity of the color developed is proportionate of the CICs present in the sample.

REAGENTS
Receptor Microplate (IC-00101) – One polystyrene microplate (12X8 wells) coated with receptors.

Anti-IgG (γ-chain) - HRP (1000101) (Goat) – One bottle containing 12 ml of anti-IgG γ chain antibody as a HRP conjugate. Purified immunoglobulin fraction conjugated to Horseradish peroxidase (HRP).

Aggregated Human γ-Globulin (AHG) – One bottle containing 1 ml of lyophilized AHG supplied at 30µg/ml. Solubilize the lyophilized AHG with one ml of wash buffer. Make eight serial dilution of 30 µg/ml standard with 1X wash buffer up to 0.23 µg/ml.

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 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 or dilute samples.

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 heparin or citrate as anticoagulants. Centrifuge at 1500 g within 30 minutes of collection. Assay immediately or store samples in aliquots.

SAMPLE SORAGE
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.

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SAMPLE PREPARATION
Serum or plasma is diluted 1:20 to 1:50 using PBS/Tween-20 (0.05% Tween). For samples having higher concentration of IgG-CIC should be diluted appropriately and assayed again so the measured optical density falls within the linear range of the standard curve. For making serial dilutions for AHG standards use 1X wash buffer provided in the kit. Always use inert material containers for AHG dilution. AHG dilutions can also be made directly in the plate wells if appropriate containers are not available.

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.

9. Add 100 µl of color development reagent and stop the reaction by addition of 50 µl of stop reagent when desired optical density is reached. It is usually 3 to 7 minutes. During the development gently tap the plate occasionally.

10. Read the plate using a microplate reader at 450 nm.

11. Plot the standard values against the known concentration and using the linear equation calculate the sample values (y = a + bX).

CALCULATION OF RESULTS
Make a spreadsheet and enter the data. Average the duplicate reading 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 AHG 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 AN 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 AHG:

<table>
<thead>
<tr>
<th>AHG µg/ml</th>
<th>OD 450</th>
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<tbody>
<tr>
<td>0.23</td>
<td>0.15</td>
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<tr>
<td>0.47</td>
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<tr>
<td>0.94</td>
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<td>1.59</td>
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<td>2.61</td>
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Slope: 0.08367
Intercept 0.19867

Use the linear portion of the curve.

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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