Circulating Immune complexes
IgM-CIC Proceptor™ ELISA Kit
Data Sheet (Product Code IC 002)

INTRODUCTION
An immune complex (IC) is formed from the integral binding of an antibody to a soluble antigen. Formation of ICs is a normal physiological process for the removal of foreign substances. However, certain patho-physiological events can lead to excessive formation and poor clearance of ICs that could lead to deposition of these ICs at various tissue sites. Circulating immune complexes (CIC) play an important role in the pathogenesis of glomerular, vascular and connective tissue lesions in several human diseases. They are involved particularly in autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).

Deposition of IgM-IC onto follicular dendritic cells (FDCs) plays a role in stimulating the onset of the adaptive humoral immune response. CIC formed by tumor antigens and immunoglobulin M (IgM) represent a novel class of biomarkers with diagnostic value for early cancer detection.

Proceptor™ (IC-002) CIC-IgM ELISA is based on a receptor interaction, which binds to the constant region of complexed immunoglobulin. The assay efficiently detects the IgM within CIC. We also offer ELISA assays for measuring IgA and IgG present in the CICs.

ASSAY PRINCIPLE
The assay is based on unique capture of CIC by ProGen’s Proprietary Receptors* isolated from lymphoid cell lines. These purified receptors* are coated onto the microtiter plate. Diluted serum samples are incubated in the wells to allow IgM-CIC present in the serum to bind with the receptors*. Nonspecific reactants are removed by washing; a polyclonal antibody to IgM µ-chain specific HRP conjugated is added to all wells and allowed to interact with the CIC. Excessive conjugate is removed by washing. After incubation a substrate (TMB) solution is added to the wells. Intensity of the color developed is proportionate to the amount of the IgM-CIC. Sample OD readings are compared with reference negative and positive controls OD readings to determine results.

REAGENTS
Receptor Microplate (IC-00102) – One Polystyrene microplate (12X8 wells) coated with receptors* specific for complexed immunoglobulin.

Anti-IgM (µ-chain) – HRP (1000102) (Goat) – One bottle containing 12 ml of anti-IgM µ chain HRP antibody as a conjugate, with stabilization solution and preservative. Purified immunoglobulin fraction conjugated to Horseradish peroxidase (HRP).

Control Serum Samples – IgM High Positive Control, 0.5 ml. IgM Low Positive Control, 0.5 ml. One vial for each control is supplied. Dilution required before use with 1X wash buffer.

Substrate Reagent (1000901) – One bottle, containing 12 ml of substrate solution TMB (tetramethylbenzidine).

Stop Reagent (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. Use IX wash buffer for washing 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 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.

*ProGen’s proprietary receptors and their use are covered under US patent 7682793 B2.
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SAMPLE PREPARATION
Serum or plasma is diluted 1:20 using 1X wash buffer. For samples having higher concentration of IgM-CIC bound should be diluted appropriately and repeat the assay so the measured optical density falls within the standard curve.

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 the samples with 1X wash buffer. We recommend 1:20 dilution of the samples.
2. Fill wells with 200 µl of 1X wash buffer. A 2-3 minutes soaking is recommended with each wash step. Aspirate wells to remove buffer. Blot the emptied wells face down on clean paper towel and tap the plate vigorously to completely remove residual wash buffer. Seal plate with plastic sealers during each incubation period.
3. Dispense 100 µl of sample per well. It is recommended to run the assay in duplicate wells.
4. Dispense standard serum samples provided as internal control in the wells and mark their location.
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.
7. Add 100 µl of anti-IgM - HRP conjugate to each well, using 8 or 12 channel pipette. Incubate the plate for ninety minutes at room temperature (20 to 25°C).
8. Repeat wash as in step 6
9. Add 100 µl of substrate solution using 8 or 12 channel pipette. Incubate for 10 ± 1 minute at room temperature (20 to 25°C).
10. Stop the reaction by addition of 50 µl of stop reagent when desired optical density is reached. During the development gently tap the plate occasionally. Make sure that no air bubbles are trapped in the wells during the color development.

11. Read the plate using a microplate reader at a wavelength of 450 nm.
12. Plot the standard values against the known concentration and using the linear equation calculate the sample values (y = a + bX).

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 reading for each, control and sample. Subtract the average zero standard optical density, if the function is available in the machine this can be done by the plate reader. Two serum samples with high and low IgM complexes are provided as a control for assay. On screening of over forty normal samples all except two samples showed OD at 450 of more than 0.4, the average OD was 0.28. Thus a value 0.50 or below is considered normal. All values above are considered high. Alternately a curve can be generated using high positive control.

PRECAUTION AND LIMITATIONS 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.

<table>
<thead>
<tr>
<th>Serum Levels of IgA-CIC</th>
<th>OD 450 nm</th>
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</thead>
<tbody>
<tr>
<td>Low IgM-CIC</td>
<td>0.4</td>
</tr>
<tr>
<td>High IgM-CIC</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Store Refrigerated
Expiry Date:
Batch No.:
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