Circulating Immune complexes IgA-CIC Proceptor™ ELISA Kit Data Sheet (Product Code IC 003)

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. Immune-complex-mediated injury is thought to play an important role in diseases such as rheumatoid arthritis, systemic lupus erythematosus, serum sickness, various infectious diseases, and malignancies. With increased appreciation of the biological and pathological significance of circulating immune complexes (CICs) have come efforts to develop appropriate techniques for identifying and measuring ICs of individual immunoglobulin isotype. Sera of patients with IgA nephropathy contain CIC composed of galactose-deficient IgA1 complexed with anti-glycan antibodies.

Proceptor™ (IC-003) CIC-IgA ELISA is based on a receptor interaction, which binds to the constant region of complexed immunoglobulin. The assays efficiently detect the IgA containing CIC. We also offer ELISA assays for IgM and IgG containing CIC.

ASSAY PRINCIPLE

The assay is based on unique capture of CIC by ProGen's Proprietary Receptors*. These purified receptors* are coated onto the microtiter plate. Diluted serum samples are incubated in the wells to allow CIC present in the sample to bind with the receptors*. Nonspecific reactants are removed by washing. A polyclonal antibody to IgA α chain specific HRP conjugated is added to each well and allowed to interact with the CIC. Excessive conjugate is removed by washing. After incubation a substrate solution (TMB) is added to each well. Intensity of the color developed in proportion to the amount of IgA-CIC. Sample OD readings are compared with reference negative and positive controls OD readings to determine results.

REAGENTS:

Receptor Microplate (IC-00103) – One Polystyrene microplate (12X8 wells) coated with receptors* specific for complexed immunoglobulin.

Anti-IgA (α-chain) – HRP (1000103) (Goat) – One bottle containing 12 ml of anti-IgA α chain HRP antibody as a conjugate with stabilization solution and preservative. Purified immunoglobulin fraction conjugated to Horseradish peroxidase (HRP).

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

Color Development Reagent (1000901): One bottle containing 12 ml of stabilized chromogen 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 -70º C for future use. Avoid repeated freeze-thaw cycles. Sample stored at -20° C can also be used.

SAMPLE PREPARATION

Serum or plasma is diluted 1:20 using 1X wash buffer. For samples having higher concentration of IgA-CIC bound should be diluted appropriately and repeat the assay so the

*ProGen's proprietary receptors and their use are covered under US patent 7682793 B2.

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. Wash the wells with 200 µl of 1X wash buffer. Dispense 100 µl of sample per well. It is recommended to run the assay in duplicate wells.
3. Dispense standard serum samples provided as internal control in the wells and mark their location.
4. Incubate the plate for ninety minutes at room temperature (20 to 25ºC).
5. 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.
6. Add 100 µl of anti-IgA - HRP conjugate to each well. Incubate the plate for ninety minutes at room temperature (20 to 25ºC).
7. Repeat wash as in step 5.
8. Add 100 µl of substrate solution to each well and watch for color development. It will take about 5-10 minute for color to develop.
9. 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.
10. Read the plate using a microplate reader at a wavelength of 450 nm.
11. 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 IgA complexes are provided as a control for assay. On screening of over forty normal samples all except two samples showed OD450 of more than 0.4, the average OD was 0.250. Thus a value 0.50 or below is considered normal. All values above are considered high. Alternately, high positive samples can be used to generate a curve.

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.

Serum Levels of IgA-CIC	*OD 450*
Low IgA-CIC High IgA-CIC	

Store Refrigerated:
Expiry Date:
Batch No.:

*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