**Circulating Immune Complexes Ig-Kappa-CIC Proceptor™ ELISA Kit Data Sheet**

**(Product Code IC 004)**

**INTRODUCTION**

Monoclonal immunoglobulin kappa (κ) or lambda (λ) free light chains (FLCs) are important markers for identifying and monitoring patients with B cell tumors. Immunoassays that measure FLCs in urine and serum have considerable clinical importance in gammopathies, myelomas, and primary systemic amyloidosis. Polyclonal FLCs concentrations are increased in autoimmune diseases such as SLE and insulin dependent diabetic mellitus (IDDM) and also in chronic inflammatory diseases such as sarcoidosis and tuberculosis. Light chains increased in the urine and cerebrospinal fluid of patients with multiple sclerosis (MS).

Techniques available for the quantitation of FLCs cannot directly determine B cell clonality. Measurement of the circulating immune complexes (CICs) composed of specific kappa (κ) chains provides a better tool for assessing B cell clonality since serum concentrations of FLCs are several orders of magnitude lower than those of the light chains bound to intact immunoglobulin.

Proceptor™ (IC-004) kappa (κ) chain ELISA is based on a receptor interaction, which binds to the constant region of complexed immunoglobulin. The assay efficiently detects kappa (κ) chains present in the CICs.

**ASSAY PRINCIPLE**

The assay is based on unique capture of CICs by ProGen’s proprietary receptors. These purified receptors are coated onto the microtiter plate. The samples at appropriate concentration are pipetted into the wells, the circulating CICs present in the serum then bind to the receptors. After washing away any unbound material, a polyclonal antibody to κ-chain is added to the wells and allowed to interact with the κ-chain in the CICs. Again the plate is washed to remove the unbound anti-κ-chain antibody. The wells are then filled with secondary antibody-enzyme reagent specific for the anti-κ-chain antibody. After incubation a substrate (TMB) solution is added to the wells. Intensity of the color developed is proportionate to the amount of κ-chain present in the CICs.

**REAGENTS**

- **Receptor Microplate (00104):** Polystyrene microplate (12X8 wells) coated with receptors specific for complexed immunoglobulins. The lanes A1:H1 and A2:H2 are coating with doubling dilution of standards from 1.95 - 250 ng/ml.
- **Anti-Ig-Kappa chain antibody (1000104) (Rabbit):** One vial containing 1.2 ml of anti-κ chain antibody (10X) (purified globulin fraction) in a stabilization solution with preservative. Dilute with 1X wash buffer before use.
- **HRP Conjugate for Kappa Chain primary antibody (4000104)-(Goat):** One bottle containing 12 ml of 1X solution of Enzyme conjugate for detection of the primary 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 7 mL of 0.25 N Sulfuric Acid.
- **Wash Buffer (1000601):** Two bottles each containing 50 ml of 10X PBS/T/ween-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 EDTA or heparin as an anticoagulant. Centrifuge at 1500 g within 30 minutes of collection.

**SAMPLE STORAGE**

Use serum or heparinized plasma samples immediately after collection or store sample preferably below -70º C for future use. Avoid

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repeated freeze-thaw cycles. Sample stored at \(-20^\circ C\) can also be used.

**SAMPLE PREPARATION**

Serum or plasma is diluted 1:20 using 1X wash buffer. For samples having higher concentration of κ-chain-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 to appropriate dilution. We recommend 1:20 dilution of the samples.
2. In the first step leave the first two rows A1-H1 and A2-H2 containing standards 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.
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 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-κ-chain antibodies (dilute one ml stock solution to a final volume of ten ml with 1X wash buffer) to each well and incubate the plates for sixty minutes at room temperature (20 to 25°C).
8. Repeat wash as in step 6.
9. Add 100 µl of HRP-Conjugate and incubate for sixty minutes at room temperature (20 to 25°C).
11. 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. During the development gently tap the plate occasionally.
12. Read the plate using a microplate reader at 450 nm.
13. Plot the standard values against the known concentration and using the linear equation calculate the sample values \((y = a + bX)\).
14. 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 reading for each standard, 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. Use linear portion of the curve for calculation.

**PRECAUTION AND LIMITATIONS OF ASSAY**

THE ASSAY IS FOR RESEARCH USE ONLY AND NOT FOR USE IN DIAGNOSTIC PROCEDURES.

- 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.
Shown a typical standard curve

<table>
<thead>
<tr>
<th>Conc. ng/ml</th>
<th>OD 450 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.95</td>
<td>0.090</td>
</tr>
<tr>
<td>3.91</td>
<td>0.119</td>
</tr>
<tr>
<td>7.81</td>
<td>0.162</td>
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<tr>
<td>15.63</td>
<td>0.304</td>
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<tr>
<td>31.25</td>
<td>0.594</td>
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<tr>
<td>62.50</td>
<td>1.012</td>
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<tr>
<td>125.00</td>
<td>1.693</td>
</tr>
<tr>
<td>250.00</td>
<td>2.458</td>
</tr>
</tbody>
</table>

Slope 0.009826
Intercept 0.19246

Use the linear portion of the curve for calculation.

The insert represents a typical standard curve; however some variation may occur due to the laboratory and user techniques.

Store Refrigerated
Batch No.: 
Expiry Date:

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