**INTRODUCTION**

Production of a kappa (κ) or a lambda (λ) light chain in association with the functional heavy chain (H), results in the expression of an immunoglobulin (Ig) receptor on the surface of immature B cells. Monoclonal immunoglobulin kappa (κ) or lambda (λ) free light chains (FLCs) are important markers for identifying and monitoring patients with B cell tumors. Immunoglobulin light chains receptor editing has been shown to have selective influences in generating autoreactive B cells in systemic lupus erythematosus (SLE). Immunoassays that measure FLCs in urine and serum have considerable clinical importance in patients with monoclonal gammapathies, 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 are also increased in the urine and cerebrospinal fluid of patients with multiple sclerosis (MS).

Techniques available for quantitation of FLCs cannot directly determine B cell clonality. We offer a new ELISA assay for measuring lambda (λ) light chain content in the circulating immune complexes (CICs). Measurement of specific lambda (λ) chains provides a better tool for assessing B cell clonality.

Proceptor™ (IC-005) lambda (λ) chain ELISA is based on a receptor interaction, which binds to the constant region of complexed immunoglobulin. The assay efficiently detects lambda (λ) chain content within the immune complexes (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 λ-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-λ-chain antibody. The wells are then filled with secondary antibody-enzyme reagent specific for the anti-λ-chain antibody. After incubation a substrate (TMB) solution is added to the wells. Intensity of the color developed is proportionate to the amount of λ-chain present in the CICs.

**REAGENTS**

**Receptor Microplate (00105):** Polystyrene microplate (12X8 wells) coated with receptors specific for complexed immunoglobulins. Lanes A1:H1 and A2:H2 are coating with doubling dilution of standards from 1.95 - 250 ng/ml.

**Anti-Ig-Lambda-chain antibody (1000105) (Rabbit):** One vial containing 1.2 ml (10X) of anti-λ chain antibody (purified globulin fraction) in a stabilization solution with preservative. Dilute with 1X wash buffer before use.

**HRP Conjugate for Lambda Chain primary antibody (5000105) (Goat):** One bottle containing 12 ml of secondary antibody conjugate against anti-lambda 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 contain 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.

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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 -70°C for future use. Avoid repeated freeze-thaw cycles. Sample stored at -20°C can also be used.

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

SAMPLE PREPARATION
Serum or plasma is diluted 1:20 using 1X wash buffer. For samples having higher concentration of the CICs 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 1500 g 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-λ-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 readings for standards, controls and samples. Subtract the average zero standard optical density; if the function is available in the machine the plate reader can do this.

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.

PRECAUTION AND LIMITATIONS OF ASSAY
THE ASSAY IS FOR RESEARCH USE ONLY AND NOT FOR USE IN DIAGNOSTIC PROCEDURES

- The samples should be 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

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plasma samples may cause variation in binding.

**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>0.0875</td>
</tr>
<tr>
<td>3.91</td>
<td>0.12</td>
</tr>
<tr>
<td>7.81</td>
<td>0.19</td>
</tr>
<tr>
<td>15.63</td>
<td>0.334</td>
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<tr>
<td>31.25</td>
<td>0.603</td>
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<tr>
<td>62.50</td>
<td>1.063</td>
</tr>
<tr>
<td>125.00</td>
<td>1.767</td>
</tr>
<tr>
<td>250.00</td>
<td>2.587</td>
</tr>
<tr>
<td>Slope:</td>
<td>0.0130</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.201</td>
</tr>
</tbody>
</table>

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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