**Protocol of serum or plasma BUN assay.v2**

This is a modified assay for quantification of blood urine nitrogen according to DetectX® BUN Colorimetric Detection Kit (K024-H1) Product Protocol.

**Sample Preparation**

1. Prepare mouse serum or plasma according to protocols described on our website.
2. Dilute serum and plasma in ddH₂O ≥ 1:10 and ≥1:20, respectively (recommended).
3. Dilute serum: Pipet 4 µl serum in 76 µl of ddH₂O in our experience (1:20).

**Standard Preparation**

Use distilled or deionized water to prepare standard from BUN standard stock (100 mg/dl)

<table>
<thead>
<tr>
<th></th>
<th>Std 1</th>
<th>Std 2</th>
<th>Std 3</th>
<th>Std 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ddH₂O (µl)</td>
<td>138.7</td>
<td>37.5</td>
<td>37.5</td>
<td>75</td>
</tr>
<tr>
<td>Addition</td>
<td>11.3 µl Stock</td>
<td>75 µl Std 1</td>
<td>37.5 µl Std 2</td>
<td>N/A</td>
</tr>
<tr>
<td>Final conc (mg/dl)</td>
<td>7.5</td>
<td>5</td>
<td>2.5</td>
<td>0</td>
</tr>
</tbody>
</table>

※ Use the standards within 2 hours of preparation.

**Procedure**

1. Run all samples and standards in duplicates.
2. Pipet 33 µl of samples or appropriate standards in a 96 well plate in duplicates.
3. Add 50 µl of Color Reagent A to each well using a repeater pipette.
4. Add 50 µl of Color Reagent B to each well using a repeater pipette.
5. Incubate at room temperature for 30 minutes.
6. Read the optical density at 450 nm.

**Calculation**

Average the duplicate OD readings for each standard and sample. Create a standard curve after subtracting the mean OD’s for the blank (Std 4). Calculate sample concentrations using the standard curve and multiplying by the dilution factor.