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 $ddH_20 \geq 1:10$ and $\geq 1:20$, respectively (recommended).
3. Dilute serum: Pipet 4 µl serum in 76 µl of ddH_2O in our experience (1:20).

Standard Preparation

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

	Std 1	Std 2	Std 3	Std 4
ddH_20 (µl)	138.7	37.5	37.5	75
Addition	11.3 µl Stock	75 µl Std 1	37.5 µl Std 2	N/A
Final conc (mg/dl)	7.5	5	2.5	0

※ 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.