Blood Urea Nitrogen (BUN) Assay Kit

Catalog No.: abx090684

Size: 100 Assays

Storage: Store all components at 4°C for up to 6 months.

Application: For quantitative detection of BUN concentrations in serum, plasma and other biological fluids.

Detection Range: 0.05 mmol/L – 1 mmol/L

Introduction: Blood Urea Nitrogen (BUN) can be used as an indicator of renal health. Urea is produced by the liver as a waste product when protein is digested and is usually cleared from the blood by the kidneys. Blood from healthy humans typically contain between 6-20 mg/dL (1.8-7.1 mmol/L) urea nitrogen. An elevated BUN concentration may be indicative of impaired kidney function.

Abbexa's BUN Assay Kit is a quick, convenient, and sensitive method for measuring BUN in biological samples with little to no pretreatment necessary. BUN concentrations can be calculated from the colorimetric readout at 525 nm.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 12 ml
4. Dye Reagent: 1 vial
5. Standard: 1 vial

Materials Required But Not Provided

1. Microplate reader (525 nm) and incubator
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Ice
7. Sonicator
8. Mortar

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Dye Reagent Solution**
  Add 6 ml of distilled water into the Dye Reagent vial and mix thoroughly to prepare the Dye Reagent Solution. Ensure that the Dye Reagent has completely dissolved prior to use.

- **Standard Solution**
  Add 1 ml of distilled water into the Standard vial and mix thoroughly, ensuring that the Standard has completely dissolved. Add 10 µl of this solution into 990 µl of distilled water to prepare the 1 mmol/L Standard Solution.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant, and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

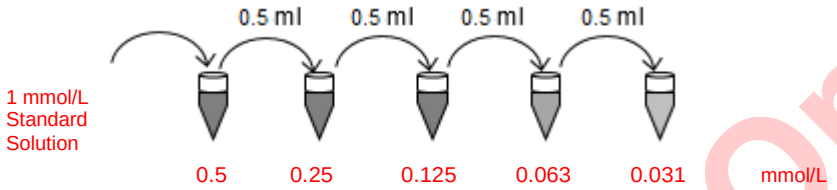
- **Liquid samples**
  Liquid samples can be used directly.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentrations within the detection range of the kit.

1. Label 5 tubes with 0.5 mmol/L, 0.25 mmol/L, 0.125 mmol/L, 0.063 mmol/L, and 0.031 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 1 mmol/L standard solution into the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set sample, standard, and blank wells and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 20 µl of Sample to each sample well.
4. Add 20 µl of distilled water to each blank well.
5. Add 20 µl of prepared standard to each standard well.
6. Add 120 µl of Reaction Buffer to each well.
7. Add 60 µl of Dye Reagent Solution to each well.
8. Tap the plate gently to mix. Incubate at 90 °C for 20 minutes.
9. Allow the plate to cool to room temperature. Read and record the absorbance at 525 nm.

C. Calculations

1. BUN concentration per mass of protein (in mg):

$$\mathbf{BUN\ (\mu mol/mg\ protein)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{C_{Protein} \times V_{Sample}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{1}}{\mathbf{C_{protein}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

2. BUN concentration per weight of sample (in g):

$$\mathbf{BUN\ (\mu mol/g)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{W \times V_{Sample}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{1}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

3. BUN concentration per thousand cells or bacteria:

$$\mathbf{BUN\ (\mu mol/10^4\ cells)} = \frac{\mathbf{C_{Standard} \times V_{Standard} \times V_{Assay}}}{\mathbf{N \times V_{Sample}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{1}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

4. BUN concentration per volume of sample (in ml):

$$\mathbf{BUN\ (\mu mol/ml)} = \frac{\mathbf{C_{Standard} \times V_{Standard}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}} = \frac{\mathbf{OD_{Sample} - OD_{Blank}}}{\mathbf{OD_{Standard} - OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (mg/ml)
$\mathbf{C_{Standard}}$	Concentration of standard (1 mmol/L = 1 µmol/ml)
N	Number of cells or bacteria ($\times 10^4$)
W	Weight of sample (g)
$\mathbf{V_{Assay}}$	Volume of assay buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.01 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.01 ml)