Blood Urea Nitrogen (BUN) Assay Kit

Catalog No.: abx090684

Size: 96 tests

Storage: Store all components at 4°C in the dark.

Application: For quantitative detection of BUN concentrations in serum, plasma, saliva, urine and milk.

Detection Range: 0.28 mmol/L – 35 mmol/L

Sensitivity: 0.09 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 580 nm.

Kit components

1. 96 well microplate
2. Enzyme: 0.05 ml
3. Enzyme Diluent: 15 ml
4. Dye Reagent: 15 ml
5. Alkaline Reagent: 15 ml
6. Standard (100 mmol/L): 2 ml
7. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (580 nm) and incubator
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Deionized water
5. Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4)
6. Timer
7. Ice
8. Sonicator
9. Mortar

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Enzyme Solution:** Dilute the Enzyme with Enzyme Diluent 301-fold (for example, add 15 ml of Enzyme Diluent to 0.05 ml of Enzyme to produce 15.05 ml of Enzyme Solution). Prepare only as much Enzyme Solution as required by the number of samples tested. Prepare immediately before assay.

- **Standards:** Label 7 tubes with 35 mmol/L, 30 mmol/L, 25 mmol/L, 20 mmol/L, 15 mmol/L, 10 mmol/L, 5 mmol/L. Add 70 µl, 60 µl, 50 µl, 40 µl, 30 µl, 20 µl, and 10 µl of Standard (100 mmol/L) to the 35 mmol/L, 30 mmol/L, 25 mmol/L, 20 mmol/L, 15 mmol/L, 10 mmol/L, 5 mmol/L tubes respectively, followed by 130 µl, 140 µl, 150 µl, 160 µl, 170 µl, 180 µl, and 190 µl of deionized water, to prepare Standard Dilutions with concentrations 35 mmol/L, 30 mmol/L, 25 mmol/L, 20 mmol/L, 15 mmol/L, 10 mmol/L, 5 mmol/L. These volumes are summarized in the following table:

Standard Dilution (mmol/L)	35	30	25	20	15	10	5
100 mmol/L Standard (µl)	70	60	50	40	30	20	10
Deionized water (µl)	130	140	150	160	170	180	190

For the blank, or 0 mmol/L standard, use pure deionized water. The volume of each standard will be 200 µl.

1. Samples

- **Serum/Plasma:** Samples should be detected directly. Keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Saliva:** Gargle with clear water 30 minutes prior to collection. Collect saliva with into a centrifuge tube, and centrifuge at 10,000 × g at 4°C for 10 minutes and take the supernatant. Keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Urine:** Collect fresh urine into a sterile container and centrifuge at 10,000 × g at 4°C for 10 minutes and take the supernatant. Keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Milk:** Collect fresh milk and centrifuge at 10,000 × g at 4°C for 10 minutes. Remove the top (white) layer, and collect the middle layer supernatant. Keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with PBS (0.01 M, pH 7.4) or Normal Saline (0.9% NaCl), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample	Dilution factor
Human serum	1
Human saliva	1
Human urine	50-70
Rat plasma	1

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Set sample, standard, and control wells and record their positions. Each sample requires a control well.
2. Add 4 µl of sample to each sample and control well.
3. Add 4 µl of prepared standard to each standard well.
4. Add 50 µl of Enzyme Solution to the standard and sample wells.
5. Add 50 µl of Enzyme Diluent to the control wells.
6. Mix with an orbital shaker for 10 seconds, then incubate at 37°C for exactly 10 minutes.
7. Add 125 µl of Dye Reagent to each well.
8. Add 125 µl of Alkaline Reagent to each well.
9. Mix with an orbital shaker for 10 seconds, then incubate at 37°C for exactly 10 minutes.
10. Read and record the absorbance at 580 nm.

C. Calculations

The standard curve can be plotted as the absorbance of each standard solution (adjusted for the blank standard) on the y-axis against the respective concentrations on the x-axis. A linear fit is recommended for the standard curve (y = ax + b).

BUN concentration per volume of sample:

$$\mathbf{BUN\ (mmol/L)} = \frac{(\mathbf{OD_{Sample}} - \mathbf{OD_{Control}}) - \mathbf{b}}{\mathbf{a}} \times \boldsymbol{f}$$

where:

OD_{Sample}	Absorbance of the sample well
$OD_{Control}$	Absorbance of the control well
a	Gradient of the standard curve y = ax + b
b	Intercept of the standard curve y = ax + b
f	Sample dilution factor prior to assay

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.