Urea (BUN) Assay Kit

Catalog No.: abx294153

Size: 100 tests

Detection Range: 0.12 mmol/L – 15 mmol/L

Sensitivity: 0.12 mmol/L

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

Application: For the detection and quantification of Urea (BUN) concentration in serum, urine, and other biological fluids.

For Reference Only

Principle of the Assay

Abbexa's Urea (BUN) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Urea (BUN) concentration. Under strong acidic conditions and heating, diacetyl oxime is converted to diacetyl, which subsequently reacts with urea to produce a red diazine chromophore, with an absorbance maximum at 520 nm. The intensity of the color is proportional to the Urea (BUN) concentration, which can then be calculated.

Kit components

1. Acidic Reagent: 40 ml
2. Standard (10 mmol/L): 1 ml
3. Oxime Reagent: 2 × 6 ml

Materials required but not provided

1. Spectrophotometer (520 nm)
2. Double-distilled water
3. Normal saline (0.9% NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. Centrifuge and centrifuge tubes
7. Vortex mixer
8. Water bath
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Acidic Working Solution:** Prepare enough Acidic Working Solution for the tubes to be tested. Mix Acidic Reagent, with double-distilled water at a ratio of 1 : 2. For example, prepare 1050 µl of Acidic Working Solution by mixing 350 µl of Acidic Reagent with 700 µl of double-distilled water. Prepare this solution immediately before use.

Note:

- Allow all reagents to equilibrate to room temperature before use.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum and plasma**: Serum and plasma samples can be tested directly.
- **Urine:** Centrifuge samples at 10,000 × g for 10 minutes at 4°C. Carefully take the supernatant for detection. Keep on ice and assay immediately or aliquot and store at -80°C for up to 1 month.
- **Saliva:** Rinse the mouth thoroughly with water and collect saliva after 30 minutes. Centrifuge samples at 10,000 × g for 10 minutes at 4°C. Carefully take the supernatant for detection. Keep on ice and assay.

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 normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human urine	45 – 60
Human plasma	1
Human serum	1
Human saliva	1
Mouse serum	1
Rat urine	45 – 60
Rabbit plasma	1

For Reference Only

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

B. Assay Procedure

Preheat a water bath to 100°C.

1. Label centrifuge tubes for each standard, blank, and sample. *Each sample requires a corresponding control. It is strongly recommended to test all tubes in duplicate*.
2. Add 20 µl of Standard (10 mmol/L) to the standard tubes.
3. Add 20 µl of double-distilled water to the blank tubes.
4. Add 20 µl of sample to the sample tubes.
5. Add 1000 µl of Oxime Reagent to all tubes.
6. Add 1000 µl of Acidic Working Solution to all tubes.
7. Seal the tubes with laboratory film and mix thoroughly using a vortex mixer.
8. Incubate the tubes in a boiling water bath for 15 minutes.
9. Coll the tubes under running water.
10. Zero the spectrophotometer with double-distilled water. Measure the OD of each tube at 520 nm using a 1 cm pathlength cuvette.

C. Calculation of Results

$$\text{Urea (mmol/L)} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}})} \times \text{F} \times C$$

where:

$\text{OD}_{\text{Sample}}$	OD value of the sample.
$\text{OD}_{\text{Standard}}$	OD value of the standard
OD_{Blank}	OD value of the blank
C	Concentration of standard
F	Dilution factor of the sample

For Reference Only

Technical Support

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