# Technical Manual

# Urea (BUN) Colorimetric Assay Kit (Urease Method)

- Catalogue Code: MAES0137
- Size: 100 Assays
- Research Use Only

# 1. Key Features and Sample Types:

## Detection method:

Colorimetric method

## Specification:

100 Assays

## Range:

0.114-30 mmol/L

## Sensitivity:

0.114 mmol/L

## Storage:

2-8°C for 6 months

## Expiry:

See Kit Label

## Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

## 2. Background:

Urea is the major final-product of protein metabolism in the body, which constitutes the clear majority of non-protein nitrogen in blood. Blood urea nitrogen come from the liver, which excreted with urine through kidney. Renal function failure, nephritis, urinary tract obstruction and so on can make the content of blood urea increased.

## 3. Intended Use:

This kit can be used to measure urea (BUN) content in animal serum, plasma, urine and milk samples.

## 4. Detection Principle:

Urea can be decomposed into ammonia ion and carbon dioxide by urease. Ammonia ion can react with phenol chromogenic agent and form a blue substance in alkaline medium, and the production of the blue substance is proportional to the urea content which can be calculated with the colorimetric assay at 580 nm.

## 5. Kit Components & Storage:

| Item | Specification | Storage |
|---|---|---|
| **Urea Standard (100 mmol/L)** | 2 mL × 1 vial | 2-8°C, 6 months |
| **Enzyme Stock Solution** | 0.1 mL × 1 vial | 2-8°C, 6 months, avoid direct sunlight |
| **Enzyme Diluent** | 30 mL × 1 vial | 2-8°C, 6 months |
| **Chromogenic Agent** | 60 mL × 2 vials | 2-8°C, 6 months, avoid direct sunlight |
| **Alkaline NaClO** | 60 mL × 2 vials | 2-8°C, 6 months, avoid direct sunlight |

### Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (580 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)

## 6. Assay Notes:

The incubation time must be 10 min after adding enzyme working solution. Therefore, it is better to make batches if there are many samples to be detected. The number of operations in a batch should be less than 20.

## 7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **urea standard working solution (10 mmol/L):** Dilute the 100 mmol/L urea standard with double distilled water at 1: 9. Store at 2-8°C for 3 days.

3. Preparation of **enzyme working solution:** Prepare fresh enzyme working solution according to the ratio of enzyme stock solution: enzyme diluent = 1:300 before use.

## 8. Sample Preparation:

**Sample requirements:** Heparin ammonium should not be used as an anticoagulant.

**1. Serum sample:**
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

**2. Plasma sample:**
Take fresh blood into the tube which has anticoagulant, centrifuge at 700-1000 g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

**3. Urine:**
Collect fresh urine and centrifuge at 10000 g for 10 min at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the urine can be stored at -80°C for a month.

**4. Saliva:**
Gargle with clear water, collect the saliva 30 min later, centrifuge at 10000 g for 10 min at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the urine can be stored at -80°C for a month.

**5. Milk:**
Collect fresh milk, centrifuge at 10000 g for 10 min at 4°C, remove the upper layer of milky white, take the middle layer supernatant and preserve on ice before detection. If not detected on the same day, the urine can be stored at -80°C for a month.

### Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

### Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.114-30 mmol/L).

The recommended dilution factor for different samples is as follows (for reference only).

| Sample Type: | Dilution Factor: |
|---|---|
| Rabbit plasma | 1 |
| Rat serum | 1 |
| Rat plasma | 1 |
| Human serum | 1 |
| Human saliva | 1 |
| Human milk | 1 |
| Human urine | 30-60 |
| Mouse urine | 30-60 |

**Note:** The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4);

## 9. Assay Protocol:

**Ambient Temperature:** 25-30°C

**Optimum detection wavelength:** 580 nm

## 10. Operation Steps:

1. **Blank tube:** Add 0.02 mL of double distilled water to the 5 mL EP tube.
   **Standard tube:** Add 0.02 mL of 10 mmol/L urea standard working solution to the 5 mL EP tube.
   **Sample tube:** Add 0.02 mL of Sample to the 5 mL EP tube.
   **Control tube:** Add 0.02 mL of Sample to the 5 mL EP tube.

2. Add 0.25 mL of enzyme working solution to blank tube, standard tube and sample tube of step 1, add 0.25 mL of enzyme diluent to control tube, mix fully with vortex mixer, incubate at 37℃ for 10 min.

3. Add 1 mL of chromogenic agent and 1 mL of alkaline NaClO, mix fully, incubate at 37°C for 10 min

4. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube with 1 cm optical path cuvette at 580 nm.

### Operation Table

| | Blank tube | Standard tube | Sample tube | Control tube |
|---|---|---|---|---|
| **Double distilled water (mL)** | 0.02 | | | |
| **Urea standard working solution (10 mmol/L) (mL)** | | 0.02 | | |
| **Sample (mL)** | | | 0.02 | 0.22 |
| **Enzyme working solution (mL)** | 0.25 | 0.25 | 0.25 | |
| **Enzyme diluent (mL)** | | | | 0.25 |
| Mix fully, incubate in 37°C for 10 min accurately. | | | | |
| **Chromogenic agent (mL)** | 1 | 1 | 1 | 1 |
| **Alkaline NaClO (mL)** | 1 | 1 | 1 | 1 |
| Mix fully and incubate in 37°C for 10 min. Set to zero with double distilled water and measure the OD values of each tube with 1 cm optical path cuvette at 580 nm. | | | | |

## 11. Calculations:

$$\text{Urea (BUN) content (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

**$\Delta A_1$:** $OD_{Sample} - OD_{Control}$
**$\Delta A_2$:** $OD_{Standard} - OD_{Blank}$
**c:** Concentration of standard (10 mmol/L urea nitrogen=280.1 mg/L)
**f:** Dilution factor of sample before test

## 12. Performance Characteristics

| | |
|---|---|
| **Detection Range** | 0.114-30 mmol/L |
| **Sensitivity** | 0.114 mmol/L |
| **Average recovery rate (%)** | 102 |
| **Average inter-assay CV (%)** | 4.7 |
| **Average intra-assay CV (%)** | 4.6 |

### Analysis

Dilute human urine with 0.9% NaCl at the ratio of 1:49, take 0.02 mL of diluted human urine, carry the assay according to the operation table.

**The results are as follows:**

The average OD value of the sample is 0.134, the average OD value of the blank is 0.010, the average OD value of the standard is 0.175, the average OD value of the control is 0.017, and the calculation result is:

$$\text{Urea content (mmol/L)} = \frac{0.134 - 0.017}{0.175 - 0.010} \times 10 \times 50$$

$$= 354.55 \text{ mmol/L}$$

## Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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