TECHNICAL MANUAL

Urea (BUN) Colorimetric Assay Kit (Urease)

- **SKU CODE:** MAES0406
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add enzyme working solution
- Add chromogenic agent and alkaline NaClO
- Measure optical density at 580 nm

2. Intended use

This kit can be used to measure urea content in serum, plasma, urine, saliva, and milk samples.

3. Detection principle

Urea can be decomposed into ammonia ion and carbon dioxide by urease. The ammonia ion reacts with amphyl to form a green substance in alkaline medium. The production of the green substance is proportional to the urea content, which can be calculated using colorimetric assay at 580 nm.

4. Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	100 mmol/L Urea Standard	1 mL x 1 vial	2 mL x 1 vial	2-8 °C, 12 months
Reagent 2	Enzyme Stock Solution	0.05 mL x 1 vial	0.1 mL x 1 vial	2-8 °C, 12 months, shading light
Reagent 3	Enzyme Diluent	15 mL x 1 vial	30 mL x 1 vial	2-8 °C, 12 months
Reagent 4	Chromogenic Agent	60 mL x 1 vial	60 mL x 2 vials	2-8 °C, 12 months, shading light
Reagent 5	Alkaline NaClO	60 mL x 1 vial	60 mL x 2 vials	2-8 °C, 12 months, shading light

5. Materials prepared by users

Instruments:

Spectrophotometer (580 nm), Micropipette, Vortex mixer, Incubator

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For small volume reagents, please centrifuge before use to obtain sufficient amounts of reagents.

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of enzyme working solution:** Before testing, prepare sufficient enzyme working solution according to the number of test wells. For example, to prepare 1505 µL of enzyme working solution, thoroughly mix 5 µL of enzyme stock solution with 1500 µL of enzyme diluent. The enzyme working solution should be prepared fresh immediately before use.
3. **Preparation of 10 mmol/L urea standard working solution:** For each well, prepare 20 µL of 10 mmol/L urea standard working solution by mixing 2 µL of 100 mmol/L urea standard with 18 µL of double distilled water. Mix well. Store at 2-8 °C for up to 3 days.

7. Sample preparation

Sample preparation:

Serum (plasma) samples: Detect directly. If not detected on the same day, serum or plasma can be stored at -80 °C for one month.

Urine: Collect fresh urine and centrifuge at 10,000 x g for 10 min at 4 °C. Take the supernatant and preserve on ice for detection. If not detected on the same day, urine can be stored at -80 °C for one month.

Saliva: Gargle with clear water, collect saliva 30 min later, centrifuge at 10,000 x g for 10 min at 4 °C. Take the supernatant and preserve on ice for detection. If not detected on the same day, saliva can be stored at -80 °C for one month.

Milk: Collect fresh milk, centrifuge at 10,000 x g for 10 min at 4 °C, remove the upper milky white layer, take the middle layer supernatant and preserve on ice for detection. If not detected on the same day, milk can be stored at -80 °C for one month.

Dilution of sample:

The recommended dilution factors for different samples are as follows (for reference only):

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). For dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. Properly dilute the sample if the color is too dark, and multiply by the dilution factor when calculating the result.
2. It is recommended to use disposable plastic tubes to avoid contamination.
3. Prepare fresh enzyme working solution for the needed amount before use. The enzyme working solution cannot be stored for a long time.
4. The enzyme stock solution has strong adhesion. It should be slowly aspirated when using a pipette.
5. The incubation time must be exactly 10 min after adding enzyme working solution. Therefore, it is better to perform batch operations if there are many samples to be detected. The number of operations in a batch should be less than 20.

9. Operating steps

1. Blank tube: Add 0.02 mL of double distilled water to a 5 mL EP tube. Standard tube: Add 0.02 mL of 10 mmol/L urea standard working solution to a 5 mL EP tube. Sample tube: Add 0.02 mL of sample to a 5 mL EP tube. Control tube: Add 0.02 mL of sample to a 5 mL EP tube.
2. Add 0.25 mL of enzyme working solution to blank tube, standard tube and sample tube from step 1. Add 0.25 mL of enzyme diluent to control tube. Mix fully with vortex mixer and incubate at 37 °C for 10 min.

3. Add 1 mL of chromogenic agent and 1 mL of alkaline NaClO to all tubes. Mix fully and 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 using a 1 cm optical path cuvette at 580 nm.

10. Calculation

For serum (plasma) and other liquid samples:

Urea content (mmol/L) = (ΔA_1 / ΔA_2) × c × f

Where:

ΔA_1: OD_{Sample} - $OD_{Control}$

ΔA_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

11. Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	3.50	16.80	22.40
%CV	5.2	4.6	4.0

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	3.50	16.80	22.40
%CV	5.4	4.8	3.9

Recovery

Three samples of high, middle, and low concentrations were tested with 6 replicates each to obtain an average recovery rate of 102%.

Recovery

Sample	Expected Conc. (mmol/L)	Observed Conc. (mmol/L)	Recovery rate (%)
Sample 1	8.5	8.4	99
Sample 2	18.5	19.4	105
Sample 3	26	26.5	102

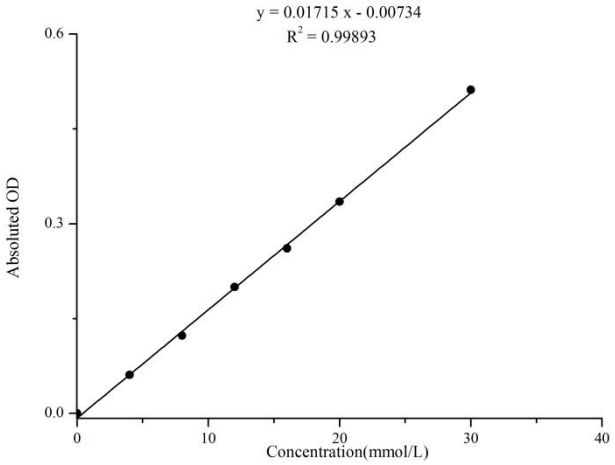
Sensitivity

The analytical sensitivity of the assay is 0.114 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

(It is not necessary to prepare a standard curve for this kit; the provided standard curve is for reference only)

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only.



Standard curve data

Concentration (mmol/L)	Average OD	Absolute OD
0	0.007	0
4	0.068	0.061
8	0.13	0.123
12	0.207	0.2
16	0.268	0.261
20	0.342	0.335
30	0.519	0.512

12. Example Analysis

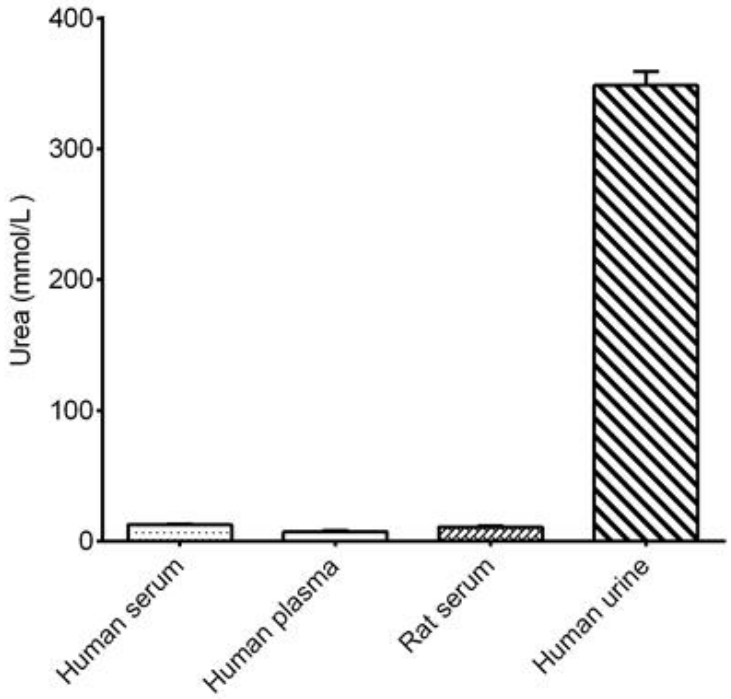
Example analysis:

Human urine was diluted with 0.9% NaCl at a ratio of 1:49. 0.02 mL of diluted human urine was taken and the assay was carried out according to the operating steps. 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:

Urea nitrogen content (mmol/L) = (0.134 - 0.017)/(0.175 - 0.010) × 10 × 50 = 354.55 mmol/L

Human serum, human plasma, rat serum, and human urine (diluted 50 times) were detected according to the protocol. The results are as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use

of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.