TECHNICAL MANUAL

Urea (BUN) Colorimetric Assay Kit (Urease)

- **SKU CODE:** MAES0136
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate with enzyme working solution
- Add chromogenic agent and alkaline NaClO
- Measure OD at 580 nm
- ![img-0.jpeg](img-0.jpeg)

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. Ammonia ion can react with amphyl and form a green substance in alkaline medium, and the production of the green substance is proportional to the urea content which can be calculated with the colorimetric assay at 580 nm.

4. Kit components & storage

Item	Component	Size 1(96 T)	Size 2 (500 Assays)	Storage
Reagent 1	100 mmol/L Urea Standard	2 mL x 1 vial	10 mL x 1 vial	2-8 °C, 12 months
Reagent 2	Enzyme Stock Solution	0.05 mL x 1 vial	0.25 mL x 1 vial	2-8 °C, 12 months, shading light
Reagent 3	Enzyme Diluent	15 mL x 1 vial	40 mL x 2 vials	2-8 °C, 12 months
Reagent 4	Chromogenic Agent	15 mL x 1 vial	40 mL x 2 vials	2-8 °C, 12 months, shading light
Reagent 5	Alkaline NaClO	15 mL x 1 vial	40 mL x 2 vials	2-8 °C, 12 months, shading light

Item	Component	Size 1(96 T)	Size 2 (500 Assays)	Storage
	Microplate	96 wells	/	No requirement
	Plate Sealer	2 pieces		

5. Materials prepared by users

Instruments:

Microplate reader (565-595 nm, optimum wavelength: 580 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

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

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, prepare 1505 µL of enzyme working solution by thoroughly mixing 5 µL of enzyme stock solution and 1500 µL of enzyme diluent. The enzyme working solution should be prepared immediately before use.
3. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 100 mmol/L urea standard with deionized water to create a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 15, 20, 25, 30, 35 mmol/L.

7. Standard dilution table

Item	Concentration (mmol/L)	100 mmol/L standard (µL)	Deionized water (µL)
①	0	0	200
②	5	10	190
③	10	20	180
④	15	30	170

Item	Concentration (mmol/L)	100 mmol/L standard (μL)	Deionized water (μL)
⑤	20	40	160
⑥	25	50	150
⑦	30	60	140
⑧	35	70	130

8. Sample preparation

Sample preparation:

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

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

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

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

Sample dilution:

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.

9. Sample dilution factors

Sample type	Dilution factor
Human serum	1
Rat plasma	1
Human saliva	1

Sample type	Dilution factor
Human urine	50-70

10. 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.

11. Operating steps

1. **Standard wells: Add 4 μL of standard solution with different concentrations to the corresponding wells.:** Sample wells: Add 4 μL of sample to the corresponding wells. Control wells: Add 4 μL of sample to the corresponding wells.
2. Add 50 μL of enzyme working solution to standard wells and sample wells, add 50 μL of enzyme diluent to control wells, mix fully with microplate reader for 10 s, then react at 37 °C for exactly 10 min.
3. Add 125 μL of chromogenic agent and 125 μL of alkaline NaClO to each well, mix fully with microplate reader for 10 s, react at 37 °C for exactly 10 min.
4. Measure the OD value of each well at 580 nm with microplate reader.

12. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absolute OD value.

3. Plot the standard curve by using absolute OD value of standard and corresponding concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

Serum (plasma) and other liquid samples:

Urea content (mmol/L) = (deltaA_{580} - b) ÷ a × f

[Note]

f: Dilution factor of sample before test.

deltaA_{580}: OD_{Sample} – $OD_{Control}$.

13. Appendix I 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 Data

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	1.50	12.70	26.00
%CV	3.3	2.7	2.4

Inter-assay Precision

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

Inter-assay Precision Data

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	1.50	12.70	26.00
%CV	4.1	4.5	4.3

Recovery

Three samples of high, middle, and low concentrations were tested 6 times in parallel to obtain an average recovery rate of 104%.

Recovery Data

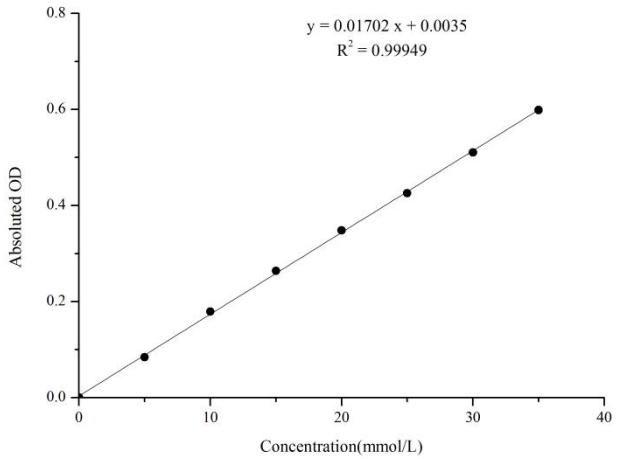
Standard	Expected Conc. (mmol/L)	Observed Conc. (mmol/L)	Recovery rate (%)
Standard 1	8.5	8.8	103
Standard 2	18	19.3	107
Standard 3	29.5	30.1	102

Sensitivity

The analytical sensitivity of the assay is 0.09 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

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	0.045	0
5	0.129	0.084
10	0.223	0.179
15	0.309	0.264
20	0.393	0.348
25	0.471	0.426
30	0.555	0.510
35	0.644	0.599

14. Appendix II Example Analysis

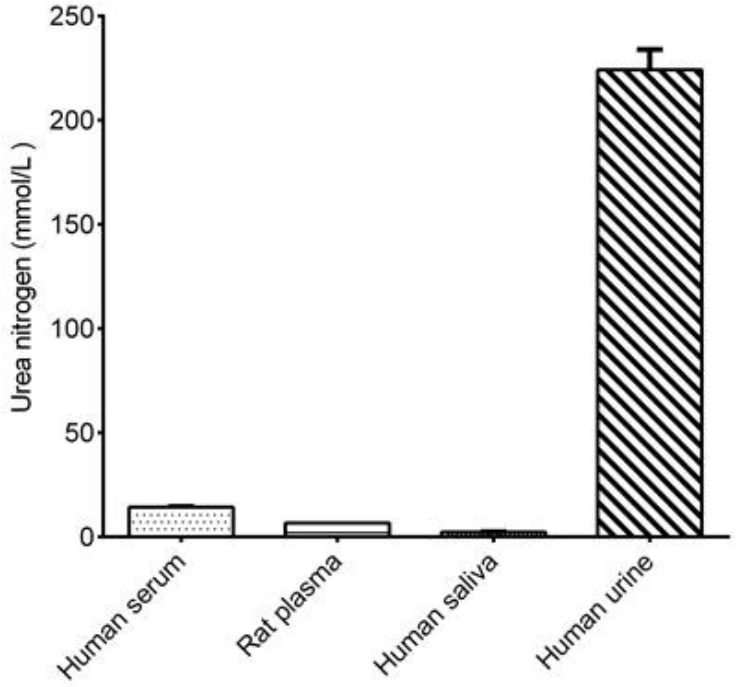
Example analysis:

Take 4 µL of rat plasma sample, carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.01702x + 0.0035, the average OD value of the sample well is 0.249, the average OD value of the control well is 0.112, and the calculation result is:

Urea content (mmol/L) = (0.249 - 0.112 - 0.0035) ÷ 0.01702 = 7.84 mmol/L

Detect human serum, rat plasma, human saliva, human urine (diluted 50 times) according to the protocol, the result is as follows:



15. 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.