

Urea Content Assay Kit (Urease Pheby, Micro Method)

U1522045

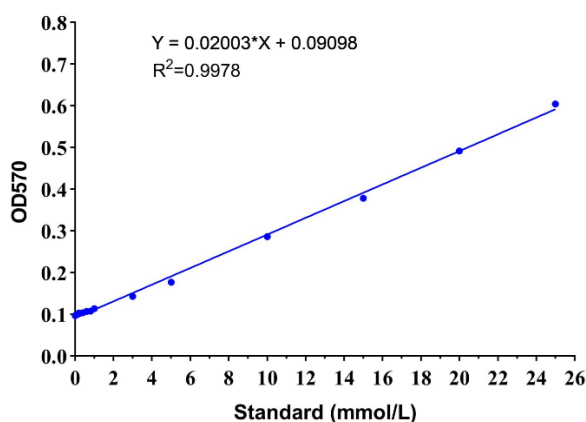
Storage -20 °C , 2-8 °C , Room temperature. Store in the dark. For detailed storage information, please refer to the kit contents. U1522045A: RT; U1522045B, U1522045C, U1522045D, U1522045E: -20°C, Store in the dark; U1522045F: -20°C, Store in the dark.

Shipping Shipped under Normal low temperature and ultra-low temperature conditions. Please store under the specified storage conditions immediately upon receipt.

Introduction

Urea, also known as carbamide, is the major nitrogen-containing end-product of protein catabolism in mammals and certain fish species. It is also the nitrogen-rich fertilizer with the highest nitrogen content currently available. Urea detection methods are generally divided into chemical and enzymatic approaches. The enzymatic method is regarded as an indirect assay: urea is first decomposed into ammonium ions by urease, and the produced ammonium ions are subsequently quantified via the Berthelot reaction. The detection principle of the Urea Assay Kit (Urease-Berthelot Microplate Method) is as follows: urease hydrolyzes urea to produce ammonia and carbon dioxide. Under alkaline conditions, ammonia reacts with phenol-related reagents to form blue indophenol. The yield of indophenol is positively proportional to urea concentration. The absorbance is measured at 560 nm using a microplate reader. This kit is applicable for determining urea (formerly referred to as blood urea nitrogen, BUN) levels in human and animal plasma, serum, urine and other samples. Urine samples are recommended to be pre-processed prior to measurement. This kit is for research-only use and shall not be applied for clinical diagnosis or other purposes.

Reference standard curve range: Follow the procedure described in the manual, measure the absorbance of urea standards at concentrations of 0, 0.2, 0.4, 0.6, 0.8, 1, 3, 5, 10, 15, 20, 25 mmol/L with a microplate reader at 570 nm, and generate the corresponding standard curve accordingly:



Note: Reference values may vary owing to reagent batch, instrument equipment, operating procedures and working-environment conditions, and are provided for reference only. Multi-point measurement using a standard curve is recommended for accurate urea quantification. Based on assay experience, deviations in the standard curve will occur when standard concentrations are below 0.2 mmol/L or above 30 mmol/L.

Component

Storage Unit No.	Storage Unit Name	Component No.	Component Name	Appearance	100 T	Storage	Quantity Per Test
U1522045NT	Urea Content Assay Kit (Urease Pheby, Micro Method) (RT Components)	U1522045A	ddH ₂ O	Colorless liquid	20 mL	Room temperature	As needed
U1522045WT	Urea Content Assay Kit (Urease Pheby, Micro Method) (2-8°C Components)	U1522045B	Urease dilution solution	Colorless liquid	3 mL	2-8°C	14 µL for 1 reaction
		U1522045C	Urea Coloration Solution	Colorless liquid	10 mL	2-8°C. Store in the dark	100 µL for 1 reaction
		U1522045D	Urea Assay Buffer	Colorless liquid	10 mL	2-8°C. Store in the dark	100 µL for 1 reaction
		U1522045E	Urea standard (100 mmol/L)	Colorless liquid	1 mL	2-8°C. Store in the dark	As needed
U1522045DT	Urea Content Assay Kit (Urease Pheby, Micro Method) (-20°C Components)	U1522045F	Urease solution	Colorless liquid	1 mL	-20°C. Store in the dark	7 µL for 1 reaction

Notes: Each component is individually packaged according to its storage temperature (RT / 2-8 °C / -20 °C) upon shipment, with differentiated temperature-controlled transportation to guarantee product performance. Orders containing materials requiring multiple temperature zones may be delivered in separate batches; please refer to the actual received goods. Upon receipt, store components separately at the temperatures specified in the component list. The shelf-life is 6 months from the date of manufacture.

Materials Required (Not Supplied)

1. Water-bath or incubator, centrifuge tubes or small test tubes.
2. Microplate reader, 96-well microplate.
3. Ammonia-free distilled water, boiling stones.

Protocol (For Reference Only)

1. Sample Preparation
 - 1) Plasma and serum: Prepare plasma and serum following routine laboratory protocols. The obtained samples can be directly used for kit measurement and stored frozen at -20°C .
 - 2) Urine: Urine samples are preferably pre-treated before detection. The pretreatment procedure is described below: Take 0.6 mL urine sample, add 0.3 g boiling stones, and top up to 15 mL with ammonia-free distilled water. Vortex repeatedly to adsorb free ammonium salts in urine. After standing, collect the diluted urine for assay; multiply the measured result by 25 for final calculation. If the urine volume is limited, reduce the volume of each reagent proportionally. For 1 mL urine sample, add 0.5 g boiling stones and make up to 25 mL with ammonia-free distilled water. Vortex repeatedly to adsorb free ammonium salts. After standing, take the diluted urine for measurement, and multiply the reading by 25.
2. Preparation of standard working solution. Dilute appropriate volume of 100 mmol/L Urea standard to prepare serial gradient standards (0, 0.2, 0.4, 0.6, 0.8, 1, 3, 5, 10, 15, 20, 25 mmol/L), which serve as standard working solutions. Alternatively, mix 100 mmol/L Urea standard with ddH₂O at a volume ratio of 1:19 to obtain 5 mmol/L urea standard working solution. Store at 4°C and use within one week.
3. Preparation of urease working solution. Mix Urease solution with Urease dilution solution at a volume ratio of 1:3 to prepare urease working solution. Store at 4°C protected from light, valid for one month.
4. Urea loading Set blank wells, standard wells and sample-testing wells according to the table below. Add solutions in the listed order and avoid bubble formation. If the urea concentration in samples is excessively high, reduce sample loading volume or appropriately dilute samples prior to measurement.

Additive (μL)	Blank Well	Standard Well	Sample-Testing Well
ddH ₂ O	1	-	-
Urea Standard (5 mmol/L)	-	1	-
Test Sample	-	-	1
Urease Working Solution	20	20	20
Mix thoroughly, incubate in a 37°C water-bath for 15 min.			
Urea Coloration Solution	100	100	100
Urea Assay Buffer	100	100	100

5. Urea measurement: Mix thoroughly, incubate in a water-bath at 37 °C for 20 min. Measure the absorbance at 570 nm using a spectrophotometer, zero the instrument with the blank well, and record the absorbance values denoted as A_{standard} and A_{sample} .
6. Calculation: Two calculation methods are available for this assay:
 - 1) Urea (mmol/L): Calculated using the standard curve and corresponding formula.
 - 2) $\text{Urea (mmol/L)} = (A_{\text{sample}} / A_{\text{standard}}) \times 5 \text{ mmol/L}$

Where:

A_{sample} : absorbance of the sample well.

A_{standard} : absorbance of the standard well.

Reference Range

Serum urea for adults: 2.9-8.2 mmol/L.

Precautions

1. Absorbance can be measured at 560 nm or 630 nm in this assay.
2. A spectrophotometer can be used in place of a microplate reader; however, reagent volumes must be adjusted accordingly and the number of detectable samples will be greatly reduced.
3. Avoid ammonium-salt-based anticoagulants, as they will cause falsely elevated results.
4. High-concentration fluoride inhibits urease activity and leads to falsely low readings.
5. Without zero-setting on the microplate reader, the OD value of the blank well is typically 0.08-0.18, and the OD value for the 5 mmol/L standard well generally ranges from 0.35-0.55.
6. Visual observation: Samples with urea concentration $\leq 1 \text{ mmol/L}$ usually show light green or light-blue color; samples $\geq 2 \text{ mmol/L}$ appear blue; samples $\geq 15 \text{ mmol/L}$ show dark-blue color. Assay accuracy is generally higher near the upper limit rather than the lower limit of detection.
7. For personal safety and health, wear a lab coat and disposable gloves during operation.