

CheKine™ Micro Urine Protein Content Assay Kit (Pyrogallol Red-Molybdate Method)

Cat #: KTB2224

Size: 48 T/48 S 96 T/96 S

	Micro Urine (UP) Content Assay Kit (Pyrogallol Red-Molybdate Method)		
REF	Cat #: KTB2224	LOT	Lot #: Refer to product label
	Applicable samples: Urine sample		
	Storage: Stored at 4°C for 12 months, protected from light		

Assay Principle

Urine Protein (UP) measurement is an important indicator for diagnosing kidney diseases and serves as a fundamental biochemical assay parameter. Elevated urinary protein levels are commonly observed in conditions such as increased glomerular permeability, impaired tubular reabsorption, or abnormal protein secretion mechanisms. The detection principle of this kit involves the reaction between pyrogallol red and sodium molybdate, forming a red-colored complex. This basic complex exhibits a maximum absorption peak around 470 nm; in an acidic environment, the pyrogallol red-molybdate complex specifically binds to positively charged basic amino acid residues (e.g., lysine, arginine) within the protein. As a result, its maximum absorption peak shifts from approximately 470 nm to around 600 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent I	24 mL	48 mL	4°C, protected from light
Standard	1 mL	1 mL	4°C

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 600 nm and 700 nm
- 96-well plate, precision pipettes, disposable pipette tips
- Centrifuge
- normal saline

Reagent Preparation

Reagent I: Ready to use as supplied; Equilibrate at room temperature before use. Store at 4°C, protected from light.

Standard: Ready to use as supplied; 10 g/L, Equilibrate at room temperature before use. Store at 4°C.

Standard preparation: Using Standard, prepare standard curve dilution as described in the table:

Num.	Standard (μL)	Normal Saline (μL)	Concentration (g/L)
Std.1	30 μL of 10 g/L	70	3
Std.2	50 μL of 3 g/L	50	1.5
Std.3	50 μL of 1.5 g/L	50	0.75
Std.4	50 μL of 0.75 g/L	50	0.375
Std.5	50 μL of 0.375 g/L	50	0.1875
Std.6	50 μL of 0.1875 g/L	50	0.09375
Std.7	50 μL of 0.09375 g/L	50	0.046875
Blank	0	50	0

Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80°C for one month.

1. Urine Sample: Centrifuge 2,000 g at room temperature (25 °C) for 10 s, then directly assay the supernatant.

Assay Procedure

1. Preheat the microplate reader to 37°C, and set the detection wavelength to 600 nm and 700 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. The standard well and the blank well only need to be tested once. The following operations are operated in the 96-well plate

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Normal Saline	5	0	0
Sample	0	0	5
Standard	0	5	0
Reagent I	300	300	300

3. Mix well, Incubate for 10 min at 37°C in the dark. The absorbance value is measured at 600 and 700 nm. The absorbance at 600 nm is denoted as A_1 ; the absorbance at 700 nm is denoted as A_2 ; The Blank Well is recorded as A_{Blank} , the standard Well is marked as $A_{Standard}$, and the test Well is marked as A_{Test} . Finally calculate $\Delta A = A_1 - A_2$; $\Delta\Delta A_{Test} = \Delta A_{Test} - \Delta A_{Blank}$, $\Delta\Delta A_{Standard} = \Delta A_{Standard} - \Delta A_{Blank}$.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If $\Delta\Delta A_{Test}$ is less than 0.01, increase the sample quantity appropriately. If $\Delta\Delta A_{Test}$ is greater than 0.8, the sample can be appropriately diluted with normal saline, the calculated result multiplied by the dilution factor.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta\Delta A_{Standard}$ as the x-axis, draw the standard curve $y=kx+b$.

2. Calculation of the concentration

Bring the $\Delta\Delta A_{\text{Test}}$ of the sample into the equation to get the y value (g/L).

Calculated by volume of liquid samples: Urine protein (g/L) = $(V_{\text{sample}} \times y) \div V_{\text{sample}} \times n = y \times n$;

Where: V_{sample} : Add 0.005 mL of sample volume to the reaction mixture; n: the sample dilution factor.

Typical Data

Typical standard curve-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

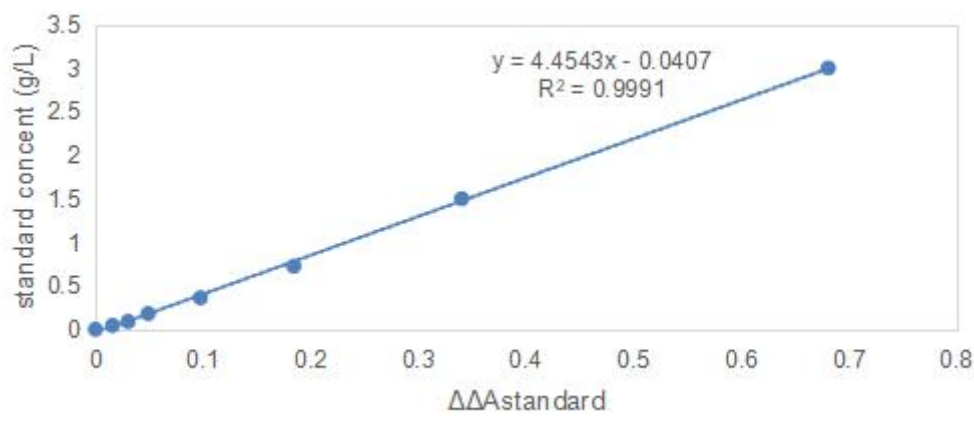


Figure 1. Standard Curve of Urinary Protein assay.

Example-1: After collecting rat urine samples and following the specified measurement procedure, the samples were analyzed using a 96-well plate. The $\Delta\Delta A$ value was calculated as $\Delta\Delta A_{\text{Test}} = \Delta A_{\text{Test}} - \Delta A_{\text{Blank}} = 0.259 - 0.220 = 0.039$; substituting this value into the standard curve yielded $y = 0.1330$. Based on the liquid volume, total urinary protein concentration (g/L) was calculated as: Urine protein (g/L) = $(V_{\text{sample}} \times y) \div V_{\text{sample}} \times n = y \times n = 0.1330$ g/L.

Recommended Products

Catalog No.	Product Name
KTB2303	CheKine™ Aspartate Aminotransferase (AST GOT) Activity Assay Kit (IFCC Rate Method)
KTB2302	CheKine™ Alanine Aminotransferase (ALTGPT) Activity Assay Kit (IFCC Rate Method)
KTB1420	CheKine™ Micro Aspartate Aminotransferase (ASTGOT) Activity Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.