PRODUCT INFORMATION & MANUAL

Urine Protein Assay Kit (Colorimetric)

NBP3-25904

For research use only.
Not for diagnostic or therapeutic procedures.

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Urine Protein Colorimetric Assay Kit

Catalog No: NBP3-25904

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 7.77 mg/L

Detection range: 7.77-100 mg/L

Average intra-assay CV (%): 2.4

Average inter-assay CV (%): 4.7

Average recovery rate (%): 105

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can measure urine protein content in urine samples.

▲Detection principle

The increase of protein concentration in urine can reflect the decrease of the reabsorption capacity of the kidney system, and can be used as an auxiliary judgment basis for kidney diseases.

The protein in urine reacts with Coomassie Blue G-250 under acidic conditions to produce chromogenic substance. The reaction system changes from brownish red to blue. The blue substance produced has the maximum absorption wavelength at 585-605 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Chromogenic Agent	24 mL × 1 vial	-20°C, 12 months, shading light
Reagent 2	500 mg/L Standard Solution	1 mL × 1 vial	-20°C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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.			

▲Materials prepared by users

Instruments

Microplate reader (585-605 nm, optimum wavelength: 595 nm)

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

⚠ Precautions

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

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. The preparation of 100 mg/L standard solution:
   Mix the reagent 2 and double distilled water at a ratio of 1:4. Prepare the fresh needed amount before use.

▲ Sample preparation

Urine sample: Detect directly.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (7.77-100 mg/L).

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

Sample type	Dilution factor
Human urine	1

Note: The diluent is double distilled water.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note:A-H, standard wells; S1-S80, sample wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 100 mg/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 30, 40, 60, 70, 80, 90, 100 mg/L. Reference is as follows:

Number	Standard concentrations (mg/L)	100 mg/L standard solution (μL)	Double distilled water (μL)
A	0	0	200
B	30	60	140
C	40	80	120
D	60	120	80
E	70	140	60
F	80	160	40
G	90	180	20
H	100	200	0

2. The measurement of samples

(1) Standard well: Add 30 μL of standard solution with different concentrations into the corresponding wells.

Sample well: Add 30 μL of sample into ample wells.

(2) Add 180 μL of eagent 1 into each wells.

(3) Mix fully with microplate reader for 5 s, stand at room temperature for 5 min Measure the OD values of each well at 595 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well
Different concentrations standard solution (μL)	30	--
Sample (μL)	--	30
Reagent 1 (μL)	180	180
Mix fully with microplate reader for 5 s, stand at room temperature for 5 min. Measure the OD values of each well at 595 nm with microplate reader.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

For urine sample:

$$\text{urine protein content (mg/L)} = (\Delta A_{595} - b) \div a \times f$$

Note:

y: $OD_{Standard} - OD_{Blank}$. ($OD_{Blank}$ is the OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA_{595}: $OD_{Sample} - OD_{Blank}$.

f: Dilution factor of sample before tested.

Appendix I Data

▲Example analysis

Take 30 μL of Human urine and carry the assay according to the operation table. The results are as follows:

standard curve: y = 0.0019 x - 0.0056, the average OD value of the control is 0.293, the average OD value of the sample is 0.399, and the calculation result is:

urine protein content (mg/L) = (0.399 - 0.297 + 0.0056) ÷ 0.0019 = 58.73 mg/L