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- Gefahrgutzuschlag
- Expressversand

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（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K252-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（585-605 nm）

Detection range：7.77-100 mg/L

Elabscience® Urine Protein Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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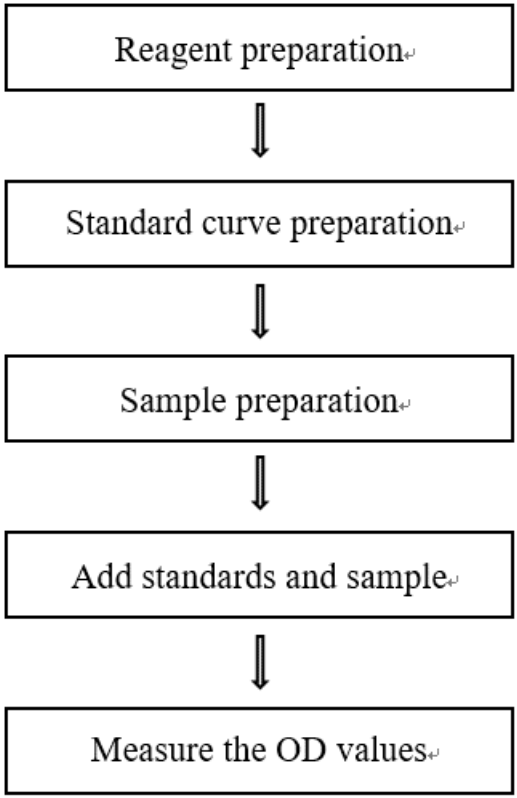
Assay summary



Intended use

This kit can be used to measure the 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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Chromogenic Agent	12 mL ×1 vial	24 mL ×1 vial	-20℃, 12 months shading light
Reagent 2	500 mg/L Standard Solution	0.5 mL ×1 vial	1 mL ×1 vial	-20℃, 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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of 100 mg/L standard solution：

Dilute 200 μL of 500 mg/L standard solution with 800 μL of double distilled water. The chromogenic agent should be prepared on spot.

③ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

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:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Standard concentration (mg/L)	**0**	**30**	**40**	**60**	**70**	**80**	**90**	**100**
100 mg/L Standard solution (μL)	0	60	80	120	140	160	180	200
Double distilled water (μL)	200	140	120	80	60	40	20	0

Sample preparation

① Sample preparation

Urine: collect fresh urine and centrifuge at 10000×g for 15 min at 4℃. Take the supernatant and preserve it on ice for detection.

② Dilution of sample

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

Sample type	Dilution factor
Human urine	1

Note: The diluent double distilled water. For the dilution of other sample types, please do pretest to confirm the dilution factor

Operating steps

① Standard well: add 30 μL of standard solution with different concentrations into the corresponding wells.
Sample well: add 30 μL of sample into sample wells.

② Add 180 μL of chromogenic agent into each wells.

③ Mix well 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

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 absoluted OD value.

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

The sample:

Urine sample:

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

[Note]

ΔA_{595}: $OD_{Sample} - OD_{Blank}$.

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/L)	15.00	42.00	85.00
%CV	2.6	2.4	2.2

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/L)	15.00	42.00	85.00
%CV	4.3	4.7	5.1

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 105%.

	standard 1	standard 2	standard 3
Expected Conc. (mg/L)	35	65	88
Observed Conc. (mg/L)	35.4	69.6	94.2
recovery rate(%)	101	107	107

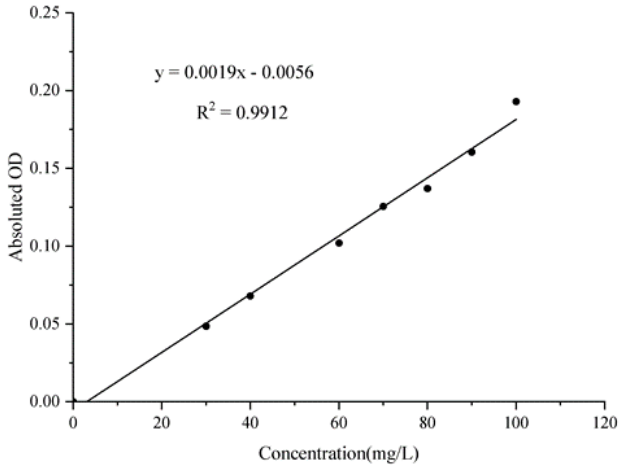
Sensitivity

The analytical sensitivity of the assay is 7.77 mg/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.

2. 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), so the standard curve and data are provided as below for reference only:

Concentration (mg/L)	**0**	**30**	**40**	**60**	**70**	**80**	**90**	**100**
OD value	0.290	0.340	0.359	0.385	0.412	0.420	0.447	0.480
	0.296	0.343	0.363	0.405	0.425	0.440	0.460	0.492
Average OD	0.293	0.342	0.361	0.395	0.419	0.430	0.454	0.486
Absoluted OD	0.000	0.049	0.068	0.102	0.126	0.137	0.161	0.193



Appendix II Example Analysis

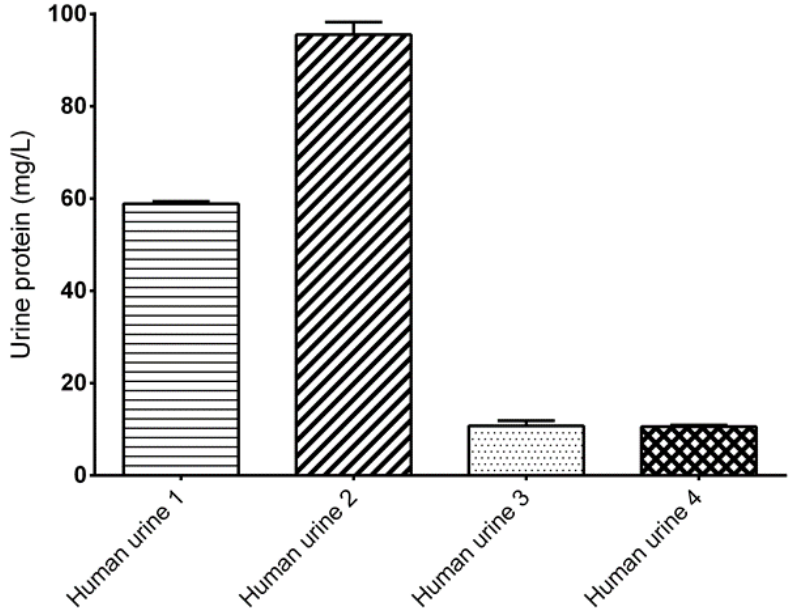
Example analysis:

Take 30 μL of human urine and carry the assay according to the operation steps. 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:

$$\text{urine protein content (mg/L)} = (0.399 - 0.293 + 0.0056) \div 0.0019 = 58.73 \text{ mg/L}$$

Determining the urine protein content of different human urine samples according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.