

## Urine Protein Content Assay Kit (Ponceau S Colorimetry)

**Note:** It is necessary to predict 2-3 large difference samples before the formal determination.

**Operation Equipment:** Spectrophotometer/Microplate reader

**Cat No:** BC5655

**Size:** 100T/96S

### Components:

**Reagent I:** Liquid 12 mL×1. Store at 2-8°C.

**Reagent I working solution:** Reagent I and distilled water are mixed by the ratio of 0.1mL: 0.9mL (1mL, 1T) to make Reagent I working solution according to sample number before use

**Reagent II:** Liquid 24 mL×1. Store at 2-8°C.

**Standard:** Liquid 1 mL×1. 5 mg/mL BSA standard solution. Store at -20°C. Add 80 μL of 5 mg/mL BSA standard solution to 920 μL of distilled water before use, mix thoroughly as 0.4 mg/mL BSA standard solution .

### Product Description:

The increase in protein content in urine indicates a weakened reabsorption of the renal system, which can usually serve as an auxiliary basis for clinical diagnosis of kidney diseases.

Adding protein precipitant and Ponceau dye to the urine sample, the protein dye complex obtained by centrifugation can be dissolved in alkaline solution. The absorbance value at 560 nm can be measured to calculate the protein content of the sample.

### Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, desk centrifuge, micro glass cuvette/96 well plate, transferpette, 2 mL EP tube, ice and distilled water.

### Procedure:

#### I. Sample preparation

- Urine and other liquid samples:** detect directly. If there is precipitation, centrifuge and take the supernatant and place it on ice for testing.

#### II. Determination

- Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 560 nm and set spectrophotometer counter to zero with distilled water.
- Add reagents in 2mL EP tube as the following:

Reagent (μL)	Test tube	Standard tube	Blank tube
Distilled water	-	-	400
Standard	-	400	-
Sample	400	-	-
Reagent I working solution	1000	1000	1000

Mix thoroughly, centrifuge at 3500 rpm for 10 minutes at room temperature, discard **all** supernatant, and leave **all** sediment.

Reagent II	200	200	200
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Mix thoroughly to dissolve the precipitate, measure the absorbance values of each tube at 560 nm, recording as  $A_T$ ,  $A_S$ , and  $A_B$ .  $\Delta A_T = A_T - A_B$ .  $\Delta A_S = A_S - A_B$ . Blank tube and standard tube only need to test once or twice.

### III. Urine protein content calculation:

Urine protein content (mg/mL) =  $\Delta A_T \times (C_S \div \Delta A_S) \times V_S \div V_T = 0.4 \times \Delta A_T \div \Delta A_S$

$C_S$ : Standard concentration, 0.4 mg/mL;

$V_S$ : Add sample volume, 0.4 mL.

#### Note:

1. After the centrifugation step is completed during the experiment, the supernatant needs to be completely removed and the precipitate must not be lost, otherwise it will affect the colorimetric results.
2. If  $\Delta A_T$  is less than 0.006 or close to  $A_B$ , it is recommended to increase added sample volume before determination. If  $\Delta A_T$  is greater than 0.4, it is recommended to dilute the sample with distilled water before proceeding with the measurement. And modify the calculation formula.

#### Experimental example:

1. Take 400  $\mu$ L experimental rat urine sample, follow the measurement steps, and use a 96 well plate to measure and calculate:  $\Delta A_T = A_T - A_B = 0.171 - 0.049 = 0.122$ ,  $\Delta A_S = A_S - A_B = 0.273 - 0.049 = 0.224$ . The result is calculated according to liquid volume:

Urine protein content (mg/mL) =  $0.4 \times \Delta A_T \div \Delta A_S = 0.217$  mg/mL.

#### Reference:

- [1] Michael A Pesce, Carl S Strande. A new micromethod for determination of protein in cerebrospinal fluid and urine[J]. Clinical Biochemistry, 1973, 19(11): 1265-1267.
- [2] Jean Dube, Joel Girouard, Pierre Leclerc. et al. Problems with the estimation of urine protein by automated assays[J]. Clinical Biochemistry, 2005, 38(5): 479-485.
- [3] Pan Ya. An analysis about the quantitative measurement of urinary protein[J]. China Health Standard Management, 2014, 5(18): 125-126.

#### Related Products:

BC5170/BC5175 Direct Bilirubin (DBIL) Content Assay Kit

BC5180/BC5185 Total Bilirubin (TBIL) Content Assay Kit

BC5590/BC5595 Free Hemoglobin (FHb) Content Assay Kit