# TECHNICAL MANUAL

# Urine Protein Colorimetric Assay Kit

- **SKU CODE:** MAES0259
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

# 1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure the OD value

# 2. Intended use

This kit can be used to measure urine protein content in urine samples.

# 3. 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 diagnostic basis for kidney diseases.

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

# 4. 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°C, 12 months, shading light |
| Reagent 2 | 500 mg/L Standard Solution | 0.5 mL × 1 vial | 1 mL × 1 vial | -20°C, 12 months, shading light |
| | Microplate | 48 wells | 96 wells | No requirement |
| | Plate Sealer | 2 pieces | 2 pieces | |

# 5. Materials prepared by users

**Instruments:**

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

# 6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **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 fresh.
3. **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 serial concentrations. The recommended dilution gradient is: 0, 30, 40, 60, 70, 80, 90, 100 mg/L.

# 7. Sample preparation

1. **Sample preparation:** Urine: collect fresh urine and centrifuge at 10,000×g for 15 min at 4°C. Take the supernatant and preserve it on ice for detection.
2. **Sample dilution:** The recommended dilution factor for human urine is 1 (no dilution). Use double distilled water as diluent. For other sample types, perform pretest to confirm the dilution factor.

# 8. 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 well.
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.

# 9. 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 absolute OD value.

3. Plot the standard curve using absolute OD value of standard as y-axis and corresponding concentration as x-axis. Create the standard curve (y = ax + b) with graph software (or Excel).

**The sample:**

Urine sample:

urine protein content (mg/L) = (ΔA_595 - b) ÷ a × f

[Note]

ΔA_595: $OD_{Sample}$ – $OD_{Blank}$.

f: Dilution factor of sample before testing.

# 10. Appendix I Performance Characteristics

Parameter:

## Intra-assay Precision

| 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

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

## Recovery

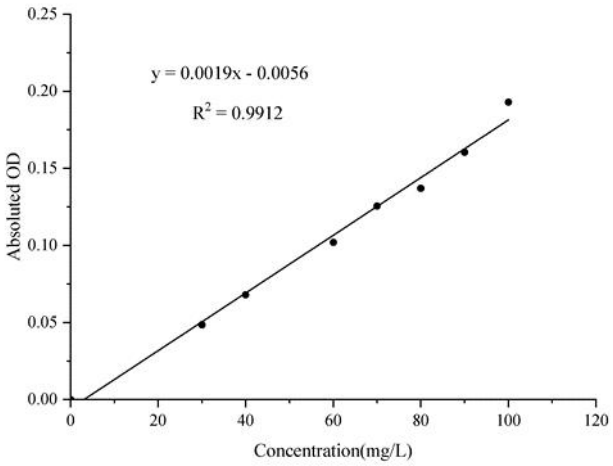
| | Standard 1 | Standard 2 | Standard 3 |
|---|---|---|---|
| Expected Conc.(mg/L) | 35 | 65 | 88 |
| Observed Conc. (mg/L) | 35.4 | 69.6 | 94.2 |
| Recovery rate(%) | 101 | 107 | 107 |

## 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.

## 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), the standard curve and data are provided below for reference only:




# 11. Appendix II Example Analysis

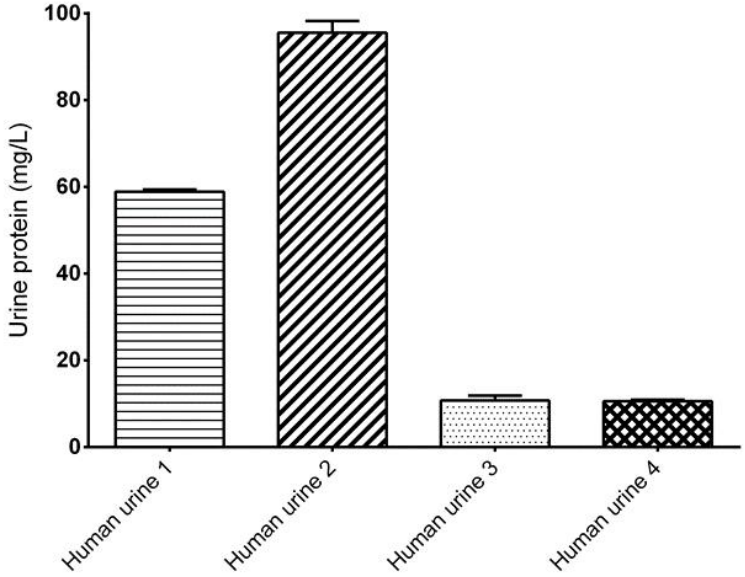
**Example analysis:**

Take 30 μL of human urine and perform the assay according to the operating steps. The results are as follows:

**Standard curve:** y = 0.0019x – 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 - 0.293 + 0.0056) ÷ 0.0019 = 58.73 mg/L

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




## 12. Statement

**1.** This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

**2.** Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

**3.** Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

**4.** If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

**5.** It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

**6.** Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

**Assay Genie 100% money-back guarantee!**

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

**Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.**