TECHNICAL MANUAL

Zinc (Zn) Colorimetric Assay Kit

- **SKU CODE:** MAES0111
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

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

2. Intended use

This kit can be used to measure zinc (Zn) content in serum, plasma, urine, and milk samples.

3. Detection principle

The zinc ion in the sample reacts with 5-Br-PADAP to produce a colored complex. The depth of color is directly proportional to the concentration of zinc ion. Zinc ion content can be calculated by measuring the OD values at 560 nm.

4. Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Size 3 (500 Assays)	Storage
Reagent 1	1.54 mmol/L Zinc Standard	0.5 mL x 1 vial	0.5 mL x 1 vial	2.5 mL x 1 vial	2-8°C, 12 months
Reagent 2	Protein Precipitator	15 mL x 1 vial	15 mL x 1 vial	40 mL x 2 vials	2-8°C, 12 months
Reagent 3	Chromogenic Agent	0.13 mL x 1 vial	0.26 mL x 1 vial	1.3 mL x 1 vial	2-8°C, 12 months, shading light
Reagent 4	Buffer Solution	13 mL x 1 vial	26 mL x 1 vial	45 mL x 3 vials	2-8°C, 12 months, shading light
	Microplate	48 wells	96 wells	/	No requirement
	Plate Sealer	2 pieces			

5. Materials prepared by users

Instruments:

Microplate reader (545-575 nm, optimum wavelength: 560 nm), Micropipettor, Vortex mixer

Reagents:

Deionized water

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of chromogenic working solution:** For each well, prepare 200 µL of chromogenic working solution by mixing 2 µL of chromogenic agent with 198 µL of buffer solution. Mix well. The chromogenic working solution should be prepared fresh immediately before use.
3. **Preparation of standard curve:** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 1.54 mmol/L zinc standard with deionized water to serial concentrations. The recommended dilution gradient is as follows: 0, 3.85, 7.7, 11.55, 15.4, 23.1, 30.8, 46.2 µmol/L.

7. Sample preparation

Sample preparation:

Do not use EDTA, citrate, or other metal chelators as anticoagulants. Do not use hemolytic samples.

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

Urine: Collect fresh urine and centrifuge at 10,000 x g for 15 min at 4°C. Take the supernatant and preserve on ice for detection. If not detected on the same day, the urine can be stored at -80°C for a month.

Milk: Collect fresh milk sample and centrifuge at 10,000 x g for 10 min at 4°C, then take the middle layer clear liquid and preserve on ice for detection. If not detected on the same day, the sample can be stored at -80°C for a month.

Dilution of sample:

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

Human urine: 1

Human serum: 1

Human milk: 1

Rat serum: 1

Note: The diluent is deionized water. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

1. The supernatant after centrifugation must be clear in the pretreatment step. Otherwise, transfer the turbid supernatant to another centrifuge tube and centrifuge again.
2. As the concentration of Zn^{2+} in serum is very low, avoid zinc contamination of vessels and reagents used in the experiment.
3. Prevent the formation of bubbles when transferring the supernatant into the microplate.
4. The sample needs to be diluted with deionized water before determination once the concentration is beyond the linear range. The result should be multiplied by the dilution factor.

9. Operating steps

1. Sample pretreatment: Mix 50 µL of sample with 50 µL of protein precipitator, and centrifuge at 13,780 x g for 10 min at 4°C, then take the supernatant for detection.
2. **Add samples and standards:** Standard well: Add 0.05 mL of standard solution with different concentrations. Sample well: Add 0.05 mL of pretreated supernatant of sample into wells.
3. Add 0.2 mL of chromogenic working solution to each well.
4. Mix thoroughly with microplate reader for 30 s and incubate for 5 min at room temperature.
5. Measure the OD value at 560 nm with microplate reader.

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

The sample:

Zn content (μmol/L) = (ΔA_560 - b) ÷ a × 2* × f

[Note]

ΔA_560: OD_{Sample} - OD_{Blank}

2*: Dilution factor of sample in pretreatment step

f: Dilution factor of sample before test

Intra-assay Precision

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

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/L)	2.50	22.50	30.50
%CV	4.3	4.2	3.5

Recovery

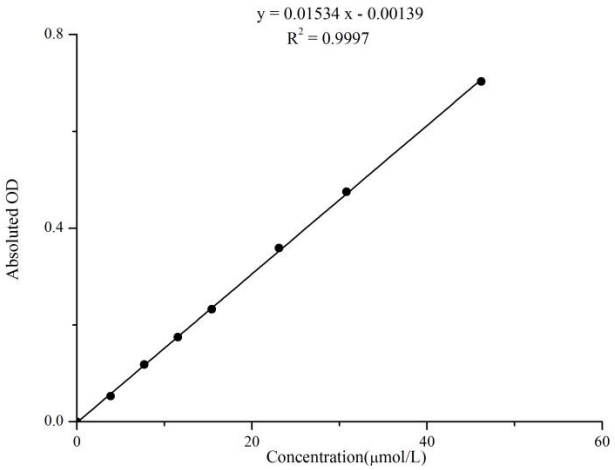
Three samples of high, middle, and low concentrations were tested with 6 replicates for each concentration to obtain an average recovery rate of 104%.

Sensitivity

The analytical sensitivity of the assay is 0.418 μmol/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 data

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

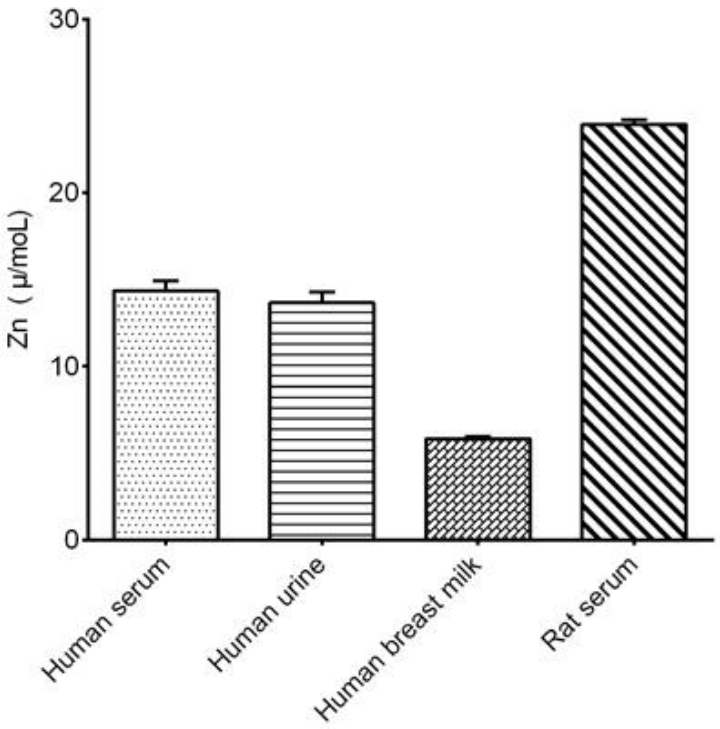
Example analysis:

Take 0.1 mL of human serum, add 0.1 mL of protein precipitator, mix thoroughly, centrifuge at 13,780 x g for 10 min at 4°C, take the supernatant and perform the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0152x + 0.0023, the average OD value of the sample is 0.217, the average OD value of the blank is 0.105, and the calculation result is:

Zn content (μmol/L) = (0.217 - 0.105 - 0.0023) ÷ 0.0152 × 2 = 14.43 μmol/L

Detect human serum, human urine, human milk, and rat serum according to the protocol, the results are 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 pretest 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.