Technical Manual

Zinc (Zn) Colorimetric Assay Kit

- **Catalogue Code: MAES0111**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.748 -46.2 μmol/L

Sensitivity:

0.418 μmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Zinc is an essential trace element for humans, animals, plants and microorganisms. It is essential for many physiological processes, such as growth and development, lipid metabolism, immune function and so on. Zinc deficiency may seriously affect the homeostasis of organism, which is associated with Parkinson's disease, hepatitis and cirrhosis, acrodermatitis enteropathica, diabetes and other diseases. Excessive zinc have toxic effects on cells, which can lead to apoptosis.

3. Intended Use

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

4. Detection Principle

The zinc ion in the sample react with 5-Br-PADAP to produce the 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.

5. Kit Components & Storage

Item	Specification	Storage
Zinc Standard (1.54 mmol/L)	0.5 mL × 1 vial	2-8°C, 6 months
Protein Precipitator	15 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent	0.26 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Buffer Solution	26 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (545-575 nm)
- Tips (10 µL, 200 µL, 1000 µL)
- EP tubes (1.5 mL, 2 mL)
- Deionized water

6. Assay Notes:

1. The supernatant after centrifugation must be clarified in in pre-treatment step. Otherwise take the turbid supernatant to another centrifuge tube and centrifuge again.

2. As the concentration of Zn^{2+} in serum is very low, prevent zinc contamination of vessels and reagents used in the experiment.

3. Prevent the formulation of bubbles when the supernatant is transferred 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.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use
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2. Preparation of **chromogenic agent working solution:** Mix the chromogenic agent and buffer solution at a ratio of 1: 99. Prepare the fresh solution before use.

8. Sample Preparation

Sample requirements: Do not use EDTA, citrate and other metal chelators as anticoagulants. Do not use hemolytic samples.

1. Serum sample:
Collect fresh blood and stand at 25°C for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4°C. Take the serum (which is the upper light yellow clarified liquid layer) and preserve on ice before detection. If not detected on the same day, the serum can be stored at -80°C for a month.

2. Plasma sample:
Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4°C. Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) and preserve on ice before detection. If not detected on the same day, the plasma can be stored at -80°C for a month.

3. Urine:
Collect fresh urine and centrifuge at 10000 g for 15 min at 4°C. Take the supernatant and preserve on ice before detection. If not detected on the same day, the urine can be stored at -80°C for a month.

4. Milk:

Collect the fresh milk sample and centrifuge at 10000 g for 10 min at 4°C, then take the middle layer clear liquid and preserve on ice before detection. If not detected on the same day, the sample can be stored at -80°C for a month.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.748 -46.2 μmol/L).

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

Sample Type:	Dilution Factor:
Human urine	1
Human serum	1
Human milk	1
Rat serum	1

Note: The diluent is deionized water.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 560 nm

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.

10. Operation Steps

The preparation of standard curve

Dilute 1.54 mmol/L zinc standard with deionized water to a serial concentration. The recommended dilution gradient is as follows: 0, 3.85, 7.7, 11.55, 15.4, 23.1, 30.8, 46.2 μmol/L.

The measurement of samples

1. The pretreatment of sample: Mix the sample and protein precipitator at a ratio of 1:1 and centrifuge at 13780 g for 10 min at 4°C, then take the supernatant for detection.

2. **Standard well:** Add 0.05 mL of standard solution with different concentrations
   Sample well: Add 0.05 mL of pretreated supernatant of sample in step 1. Add 0.2 mL of chromogenic agent working solution to each well of step 2.

3. Mix fully with microplate reader for 30 s and stand for 5 min at room temperature.

4. Measure the OD value at 560 nm with microplate reader.

Operation Table

	Standard well	Sample well
Zinc standard solution with different concentrations (mL)	0.05	
Pretreated supernatant of sample (mL)		0.05
Chromogenic agent working solution (mL)	0.2	0.2
Mix fully with microplate reader for 30 s and stand for 5 min at room temperature. Measure the OD value at 560 nm with microplate reader.		

11. Calculations

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.

Serum (plasma) and other liquid sample:

$$\text{Zn content (μmol/L)} = (\triangle A_{560}\text{-b}) \div a \times 2^{*} \times f$$

y: $OD_{Standard}$ - OD_{Blank} (OD_{Blank} is the change of 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
$\triangle A_{560}$: OD_{Sample} - OD_{Blank}
2*: Dilution factor in the step of pretreatment of sample
f: Dilution factor of sample before test

12. Performance Characteristics

Detection Range	0.748 -46.2 μmol/L
Sensitivity	0.418 μmol/L
Average recovery rate (%)	104
Average inter-assay CV (%)	4.0
Average intra-assay CV (%)	2.7

Analysis

Take 0.1 mL of human serum, add 0.1 mL of protein precipitator, then mix fully, centrifuge at 13780 g for 10 min at 4°C, take the supernatant, carry the assay according to the operation table.

The results are as follows:

Standard curve: y = 0.0152 x + 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:

$$\text{Zn content (μmol/L)} = (0.217 - 0.105 - 0.0023) \div 0.0152 \times 2$$

$$= 14.43\ \mu\text{mol/L}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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