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

**Catalog No：E-BC-K137-M**

**Specification：48T(32 samples)/96T(80 samples)/ 500Assays(484 samples)**

**Measuring instrument：Microplate reader（545-575 nm）**

**Detection range：0.748 -46.2 μmol/L**

# **Elabscience®** Zinc (Zn) 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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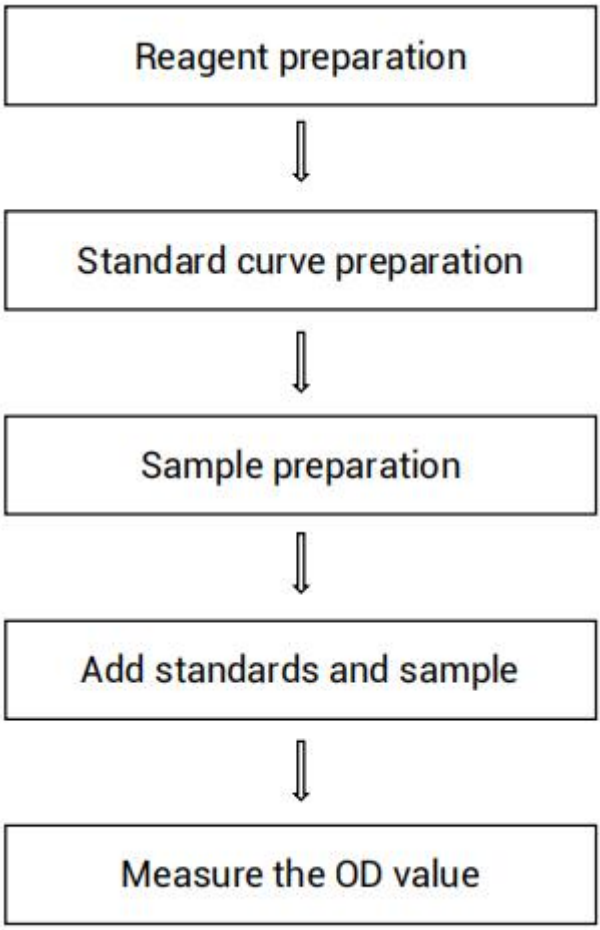
## Assay summary



## Intended use

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

## 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.

## Kit components & storage

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

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 (545-575 nm, optimum wavelength: 560 nm),

Micropipettor, Vortex mixer

**Reagents:**

Deionized water

## Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of chromogenic working solution：

For each well, prepare 200 uL of chromogenic working solution (mix well 2 μL of chromogenic agent and 198 μL of buffer solution). The chromogenic working solution 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 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. Reference is as follows:

| Item | ① | ② | ③ | ④ | ⑤ | ⑥ | ⑦ | ⑧ |
|---|---|---|---|---|---|---|---|---|
| **Concentration (μmol/L)** | **0** | **3.85** | **7.7** | **11.55** | **15.40** | **23.10** | **30.8** | **46.2** |
| **1.54 mmol/L standard (μL)** | 0 | 2.5 | 5 | 7.5 | 10 | 15 | 20 | 30 |
| **Deionized water (μL)** | 1000 | 997.5 | 995 | 992.5 | 990 | 985 | 980 | 970 |

## Sample preparation

**① Sample preparation**

Do not use EDTA, citrate and 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 10000×g for 15 min at 4°C. Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80°C for a month.

**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 it on ice for detection. If not detected on the same day, the sample can be stored at -80°C for a month.

**② Dilution of sample**

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. For the dilution of other sample types, please do pretest to confirm the dilution factor.

## The key points of the assay

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

② As the concentration of Zn2+ in serum is very low, avoid 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.

## Operating steps

① The pretreatment of sample: mix 50 μL of sample and 50 μL of protein precipitator, 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 into wells.

③ Add 0.2 mL of chromogenic working solution to each well.

④ Mix fully with microplate reader for 30 s and stand for 5 min at room temperature.

⑤ Measure the OD value at 560 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**:

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

**[Note]**

$\Delta A_{560}$: $OD_{Sample} - OD_{Blank}$

2*: Dilution factor of sample in pretreatment step.

f: Dilution factor of sample before test.

# 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 (μmol/L)** | 2.50 | 22.50 | 30.50 |
| **%CV** | 2.9 | 2.5 | 2.7 |

**Inter-assay Precision**

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

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

**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 104%.

| | Standard 1 | Standard 2 | Standard 3 |
|---|---|---|---|
| **Expected Conc. (μmol/L)** | 6.12 | 14.05 | 25.7 |
| **Observed Conc. (μmol/L)** | 6.4 | 14.3 | 27.0 |
| **Recovery rate (%)** | 105 | 102 | 105 |

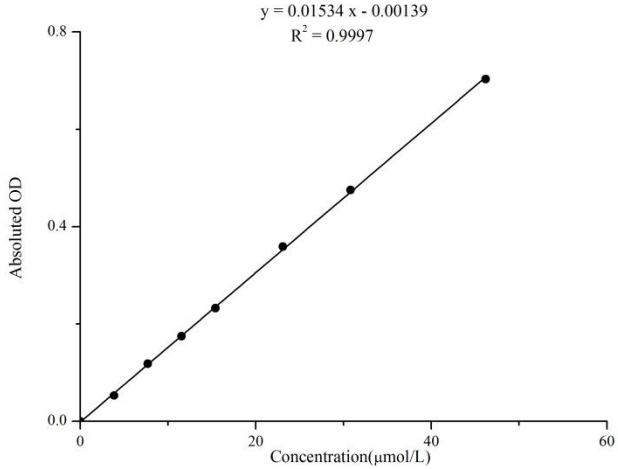
**Sensitivity**

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.

**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 (μmol/L)** | **0** | **3.85** | **7.7** | **11.55** | **15.4** | **23.1** | **30.8** | **46.2** |
|---|---|---|---|---|---|---|---|---|
| **Average OD** | 0.116 | 0.169 | 0.234 | 0.291 | 0.348 | 0.475 | 0.591 | 0.819 |
| **Absoluted OD** | 0 | 0.053 | 0.118 | 0.175 | 0.232 | 0.359 | 0.475 | 0.703 |



## Appendix II Example Analysis

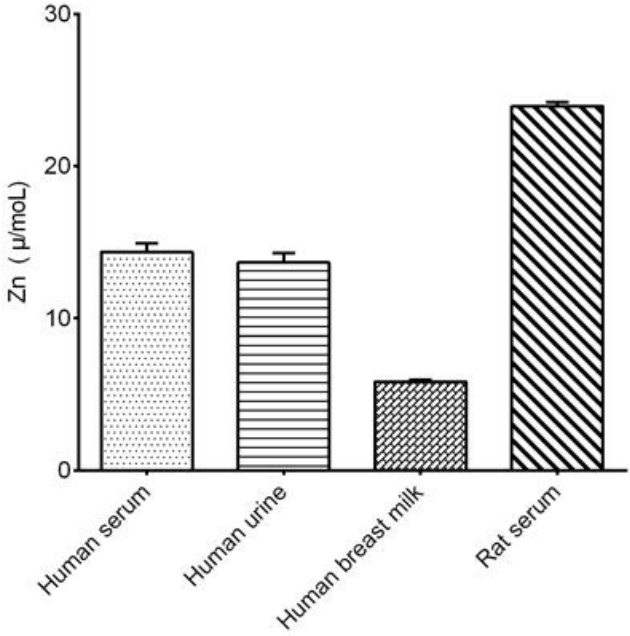
**Example analysis：**

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 and carry the assay according to the operation steps. 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:

$$\text{Zn content (μmol/L)} = (0.217 - 0.105 - 0.0023) \div 0.0152 \times 2 = 14.43\ \mu\text{mol/L}$$

Detect human serum, human urine, human milk, rat serum 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.