Zinc Assay Kit

Catalog No.: abx298876

Size: 96 tests

Detection Range: 0.748 µmol/L – 46.2 µmol/L

Sensitivity: 0.418 µmol/L

Storage: Store at 2-8 °C. store the Chromogenic Reagent and Buffer Solution in the dark.

Application: For detection and quantification of Zinc concentration in serum, plasma, urine, and milk.

Introduction

Zinc is an essential metal ion that is often used as a cofactor for many enzymes. Zinc is involved in many processes including signal transduction, gene expression, apoptosis regulation and synaptic plasticity.

Abbexa's Zinc Assay Kit is a quick, convenient, and sensitive method for measuring and calculating zinc concentrations. The reaction produces a colored compound with an absorbance maximum at 560 nm. The intensity of the color is proportional to the concentration of zinc, which can then be calculated.

Kit components

1. 96-well microplate
2. Protein precipitator: 15 ml
3. Chromogenic reagent: 0.26 ml
4. Buffer solution: 26 ml
5. Zinc standard (1.54 mmol/L): 0.5 ml
6. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (560 nm)
2. Deionized water
3. Pipette and pipette tips
4. Vials/tubes
5. Centrifuge
6. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hour. Centrifuge at approximately 2000 × g for 15 minutes at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 minutes at 1000-2000 x g at 4°C, within 30 minutes of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Urine:** Collect fresh urine into a sterile container, then centrifuge at 10,000 × g at 4°C for 15 minutes. Take the supernatant, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Milk:** Collect fresh milk into a sterile container, then centrifuge at 10,000 × g at 4°C for 15 minutes. Take the clear middle layer liquid, keep on ice and assay immediately, or aliquot and store at -80°C for up to 1 month.

We recommend carrying out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with deionized water, then carry out the assay procedure. 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:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

2. Reagents

- **Chromogenic reagent working solution:** Dilute the Chromogenic reagent 1:99 with Buffer solution. Prepare 200 µl of the chromogenic working solution for each well (2 µl of chromogenic reagent and 198 µl of buffer solution). Prepare immediately before carrying out the assay.
- **Standard Dilutions:** Label 8 tubes with 0, 3.85, 7.79, 11.55, 15.4, 23.10, 30.8, and 46.20 µmol/L. Dilute the 1.54 mmol/L Zinc standard solution with deionized water as summarized in the following table:

Concentration (µmol/L)	**0**	**3.85**	**7.79**	**11.55**	**15.40**	**23.10**	**30.08**	**46.20**
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**

For the blank, or 0 µmol/L standard, use pure deionized water. The volume of each standard will be 1000 µl.

B. Assay Procedure

1. **Pretreatment of samples:** Mix the sample with Protein precipitator at 1:1 ratio. Mix fully, centrifuge at 13,780 × g for 10 minutes at 4°C, and then take the supernatant to carrying out the assay.

2. **Chromogenic Reaction:**

2.1. Set the Standard and Sample wells on the well-plate.
2.2. Add 50 µl of prepared standards to the Standard wells.
2.3. Add 50 µl of sample to the Sample wells.
2.4. Add 200 µl of Chromogenic reagent working solution to each well.
2.5. Mix fully and allow to stand at room temperature for 5 minutes.
2.6. Measure the OD values at 560 nm with a microplate reader.

C. Calculation of Results

The standard curve can be plotted as the absolute OD_{560} of each standard solution (y) vs. the respective concentration of the standard solution (x). A linear fit is recommended for the standard curve ($y = ax + b$). The Zinc concentration of the samples can be interpolated from the standard curve.

Zinc concentration per L of sample:

$$\text{Zn content (µmol/L)} = \frac{\Delta A_{560} - b}{a} \times 2 \times \text{f}$$

where:

ΔA_{560}	OD value of the sample ($OD_{Sample} - OD_{Blank}$)
a	gradient of the standard curve (linear fit)
b	y-intercept of the standard curve (linear fit)
2	dilution factor of sample in pretreatment step
f	dilution factor of the sample before carrying out the assay