Product Manual

Zinc Assay Kit (Colorimetric)

Catalog Number

MET-5138 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Zinc is an essential trace metal involved in a multitude of biological pathways including cell growth and differentiation, nucleic acid metabolism, signal transduction, and neurological functions. It binds a variety of proteins and acts as a cofactor in many enzymes such as the carbon dioxide regulator, carbonic anhydrase, and the peptide hydrolyzing enzyme, carboxypeptidase. Zinc is important to many enzymatic reactions, transcription factors, and structural proteins. Over 300 known enzymes contain zinc in their catalytic domains. The "zinc finger" motif is prevalent in more than 20 classes of distinct modules that interact with lipids, proteins, and nucleic acids. The Lewis acid properties of zinc allow it to stabilize negative charges in catalytic processes. Extracellular release of zinc functions as a signaling mediator in endocrine, paracrine, and autocrine systems.

Zinc is the second most prevalent trace element in the body and exists at ~2-3 grams. Total normal cellular zinc concentrations range between tens to hundreds of micromoles per liter. Zinc deficiency results in many clinical manifestations including loss of appetite, impaired immunity, growth retardation, weight loss, poor wound healing, diarrhea, and mental fatigue. Disease states associated with zinc malabsorption include chronic liver disease, chronic kidney disease, sickle cell disease, diabetes, and gastrointestinal disorders.

Cell Biolabs' Zinc Assay Kit is a simple colorimetric assay that measures the total amount of zinc present in biological samples in a 96-well microtiter plate format. Samples are deproteinized to release bound zinc. Free zinc forms a complex with the color reagent to produce a bright red color, which is then measured with a standard 96-well spectrophotometric microtiter plate reader. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, zinc standards, and unknown samples. Sample zinc concentrations are determined by comparison with a known zinc standard. The kit has a detection sensitivity limit of ~0.4 µM zinc.

Related Products

1. MET-5121: Calcium Assay Kit
2. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
3. STA-340: OxiSelect™ Superoxide Dismutase Activity Assay
4. STA-347: OxiSelect™ In Vitro ROS/RNS Assay Kit (Green Fluorescence)
5. STA-360: OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit

Kit Components

1. Color Reagent (Part No. 51381B): One 16 mL amber bottle
2. Zinc Reagent (Part No. 51382B): One 4 mL amber bottle
3. Zinc Standard (Part No. 51383B): One 100 µL vial at 10 mM concentration
4. Deproteinizing Solution (Part No. 51384B): One 10 mL amber glass bottle

Materials Not Supplied

1. Distilled or deionized water
2. Hydrochloric acid
3. Standard 96-well clear microtiter plate

Storage

Upon receipt, store the kit components at 4°C.

Preparation of Reagents

Important Note: Glassware and synthetic rubber may contain large amounts of zinc. In order to avoid zinc contamination of samples, glassware should be washed thoroughly with dilute hydrochloric acid (~1 Normal), rinsed with distilled water, and dried prior to use. Use parafilm® in place of butyl rubber stoppers when needed. Avoid chelators such as EDTA since they will sequester zinc levels.

- Reaction Reagent: Warm the Color Reagent and the Zinc Reagent to room temperature prior to use. Add 1 part Zinc Reagent to 4 parts Color Reagent. Mix to homogeneity. Each sample and standard require 200 µL of Reaction Reagent. Prepare only what is needed based on the number of assays employed. See Table 1 below for examples of Reaction Reagent preparation based on the number of assays employed. The Reaction Reagent is stable for 4 days at room temperature or 2 weeks at 4ºC when protected from light.

Color Reagent (mL)	Zinc Reagent (mL)	Number of Assays (200 µL/well)
16	4	**100**
8	2	**50**
4	1	**25**

Table 1. Preparation of Reaction Reagent

Preparation of Samples

Note: Samples should be deproteinized to release protein-bound zinc into its free form.

- Tissue lysates/homogenates: Homogenize tissue samples in deionized water on ice. Add the Deproteinizing Solution to tissue lysate samples at a 1:1 ratio by adding 50 µL of Deproteinizing Solution to 50 µL of sample. The proteins will immediately precipitate. Vortex to mix well and incubate for 5 minutes. Centrifuge the samples at 14,000 x *g* for 5-10 minutes to pellet the precipitate. Carefully remove the liquid for testing. The supernatant may be assayed undiluted or diluted as necessary in deionized water.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in deionized water. Homogenize or sonicate the cells on ice. Centrifuge to remove debris at 14000 x *g* for 15 minutes at 4°C. Add the Deproteinizing Solution to tissue lysate samples at a 1:1 ratio by adding 50 µL of Deproteinizing Solution to 50 µL of sample. The proteins will immediately precipitate. Vortex to mix well and incubate for 5 minutes. Centrifuge the samples at 14,000 x *g* for 5-10 minutes to pellet the precipitate. Carefully remove the liquid for testing. The supernatant may be assayed undiluted or diluted as necessary in deionized water.
- Cerebrospinal fluid: Add the Deproteinizing Solution to tissue lysate samples at a 1:1 ratio by adding 50 µL of Deproteinizing Solution to 50 µL of sample. The proteins will immediately precipitate. Vortex to mix well and incubate for 5 minutes. Centrifuge the samples at 14,000 x *g* for 5-10 minutes to pellet the precipitate. Carefully remove the liquid for testing. The supernatant may be assayed undiluted or diluted as necessary in deionized water.
- Urine: Urine samples need to be acidified to pH 3-4 to dissolve sediment which absorbs zinc. Use approximately 1-2 drops of concentrated HCl (12 N) per 15 mL of urine as a guide. The supernatant may be assayed undiluted or diluted as necessary in deionized water.

- Serum or Plasma: Add the Deproteinizing Solution to serum or plasma at a 1:1 ratio. Add 50 µL of Deproteinizing Solution to 50 µL of sample. The proteins will immediately precipitate. Vortex to mix well and incubate for 5 minutes. Centrifuge the samples at 14,000 x *g* for 5-10 minutes to pellet the precipitate. Carefully remove the liquid for testing. The supernatant may be assayed undiluted or diluted as necessary in deionized water.

Note: All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.

Preparation of Standard Curve

Prepare fresh Zinc standards before use by diluting in deionized water according to Table 2. Zinc standards may also be prepared in a 1:1 Deproteinizing Solution/deionized water solution.
Note: 1 µM Zinc = 6.54 µg/dL.

Standard Tubes	10 mM Zinc Standard (µL)	Deionized Water (µL)	Zinc (µM)	Zinc (µg/dL)
1	10	990	100	654
2	250 of Tube #1	250	50	327
3	250 of Tube #2	250	25	163.5
4	250 of Tube #3	250	12.5	81.7
5	250 of Tube #4	250	6.25	40.8
6	250 of Tube #5	250	3.13	20.4
7	250 of Tube #6	250	1.56	10.2
8	250 of Tube #7	250	0.78	5.1
9	250 of Tube #8	250	0.39	2.55
10	0	250	0	0

Table 2. Preparation of Zinc Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50 µL of each Zinc Standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 200 µL of Reaction reagent to each well and mix.
4. Incubate the reaction plate for 10-60 minutes protected from light at room temperature.
5. Read the plate with a spectrophotometric microplate reader at 540-570 nm.
6. Subtract the blank standard value from the other standard and unknown sample values to obtain the net absorbance values.

Example of Results

The following figures demonstrate typical Zinc Assay Kit results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

Figure 1: Example Zinc Standard Curve (100 µM to 0 µM) in a 96-Well Microtiter Plate (0.78 and 0.39 µM points omitted).

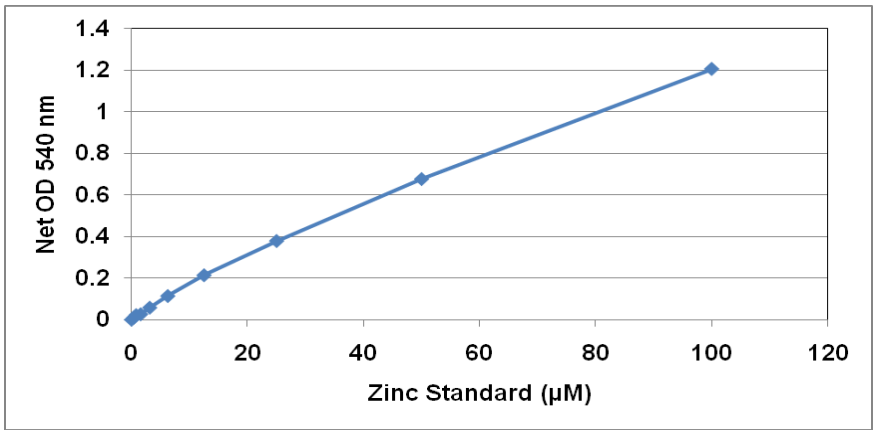

Figure 2: Example Zinc Standard Curve.

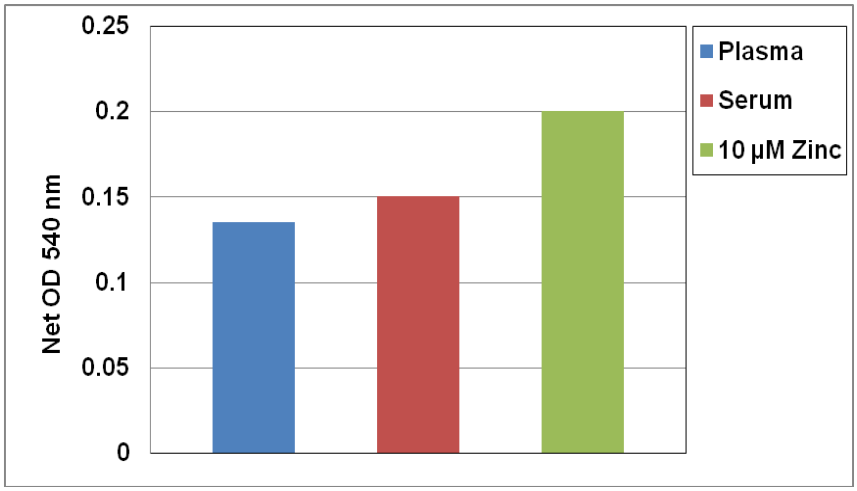

Figure 3: Zinc Detection in Normal Human Plasma or Serum. Zinc was measured in human samples. Samples were tested after deproteinizing according to the Preparation of Samples section and Assay Protocol.

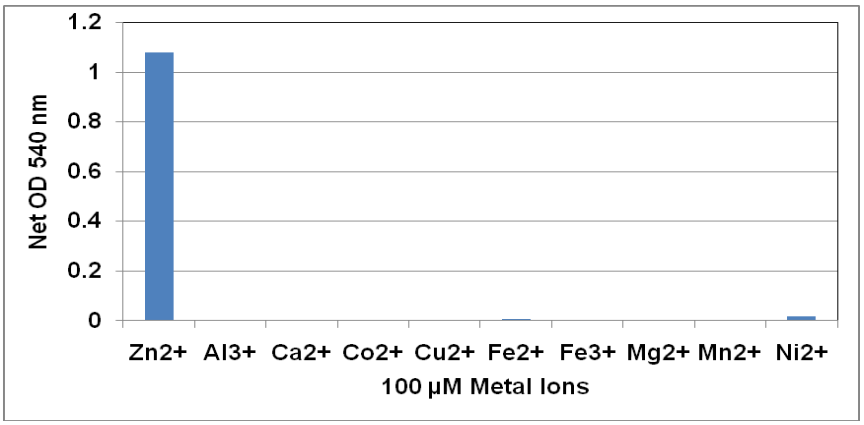

Figure 4: Metal Ion Interference Measurement. 100 µM of various metal ions were tested for cross reactivity. Samples were tested according to the Assay Protocol.

References

1. Fuse, H., et al. (1999) *Int. Urol. Nephrol.* **31**: 401–408.
2. Grüngreiff, K., et al. (2016) *Ann. Hepatol.* **15**: 7–16.
3. Powell, S.R. (2000) *J. Nutr.* **130**: S1447–S1454.
4. Prasad, A.S. (2009) *J. Am. Coll. Nutr.* 2009 **28**: 257–265.
5. Wessels, I., et al. (2017) *Nutrients* **9**: 1286.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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