# Zinc Assay Kit (Colorimetric)

Zinc Assay Kit (Colorimetric) is a detection kit for the quantification of Zinc in serum, plasma, urine, saliva, food and beverage.

Catalog number: ARG82199

Package: 250 tests

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## INTRODUCTION

Zinc is a chemical element with the symbol Zn and atomic number 30. Zinc is a slightly brittle metal at room temperature and has a silvery-greyish appearance when oxidation is removed. It is the first element in group 12 (IIB) of the periodic table. In some respects, zinc is chemically similar to magnesium: both elements exhibit only one normal oxidation state (+2), and the $Zn^{2+}$ and $Mg^{2+}$ ions are of similar size. Zinc is the 24th most abundant element in Earth's crust and has five stable isotopes. The most common zinc ore is sphalerite (zinc blende), a zinc sulfide mineral. The largest workable lodes are in Australia, Asia, and the United States. Zinc is refined by froth flotation of the ore, roasting, and final extraction using electricity (electrowinning).

Zinc is an essential mineral, including to prenatal and postnatal development. Zinc deficiency affects about two billion people in the developing world and is associated with many diseases. In children, deficiency causes growth retardation, delayed sexual maturation, infection susceptibility, and diarrhea. Enzymes with a zinc atom in the reactive center are widespread in biochemistry, such as alcohol dehydrogenase in humans. Consumption of excess zinc may cause ataxia, lethargy, and copper deficiency. [Provide by Wikipedia: Zinc]

## PRINCIPLE OF THE ASSAY

This Zinc Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of Zinc present in serum, plasma, urine, saliva, food and beverage. This assay is designed to measure zinc directly in biological samples without any pretreatment. The present method utilizes a chromogen that forms a colored complex specifically with zinc. The intensity of the color, measured at O.D. 425 nm, is directly proportional to the zinc concentration in the sample.

## MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped at room temperature. Store Reagents B and C at -20°C and other components at 4°C. Shelf life: 6 months after receipt.

| Component | Quantity | Storage information |
|---|---|---|
| Reagent A | 50 mL | 4°C |
| Reagent B | 1 mL | -20°C |
| Reagent C | 1 mL | -20°C |
| EDTA (100 mM) | 1 mL | 4°C |
| Standard (50 µM) | 1 mL | 4°C |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at O.D. 425 nm
- Clear flat-bottom 96 well microplate
- Deionized or Distilled water
- Pipettes, pipette tips and Multichannel micropipette reservoir

## TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- Because the shift in the peak wavelength (from 413 nm to 425 nm) is very small, the color change is not visually evident. Physiological concentrations of other metal ions do not interfere. $Zn^{2+}$ chelators (E.g., EDTA, EGTA) should be avoided during sample preparation.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes at 4°C. Prior to assay, dilute serum samples 5-fold (n = 5) in deionized water.

Plasma: Collect blood with heparin or citrate and centrifuge at 2000 x g for 10 minutes at 4°C. Prior to assay, dilute plasma samples 5-fold (n = 5) in deionized water.

Other liquid biological sample: If present, precipitates should be removed by filtration or centrifugation with centrifuge.

Note:

- Physiological concentrations of other metal ions do not interfere. $Zn^{2+}$ chelators (E.g., EDTA, EGTA) should be avoided during sample preparation.

## REAGENT PREPARATION

- **Working Reagent:** for each well, mixing 200 µL of Reagent A, 4 µL of Reagent B and 4 µL of Reagent C. Vortex Reagents B and C before assay.
- **Standard:** Dilute Standard as follows.

| Standard tube | $Zn^{2+}$ (µM) | Distilled water (µL) | Standard, 50 µM (µL) |
|---|---|---|---|
| S1 | 10.0 | 80 | 20 |
| S2 | 7.5 | 85 | 15 |
| S3 | 5.0 | 90 | 10 |
| S4 | 2.5 | 95 | 5 |
| S5 | 0 | 100 | 0 |

## ASSAY PROCEDURE

Equilibrate reagents to room temperature. Vortex Reagents B and C before assay. Briefly centrifuge tubes before use.

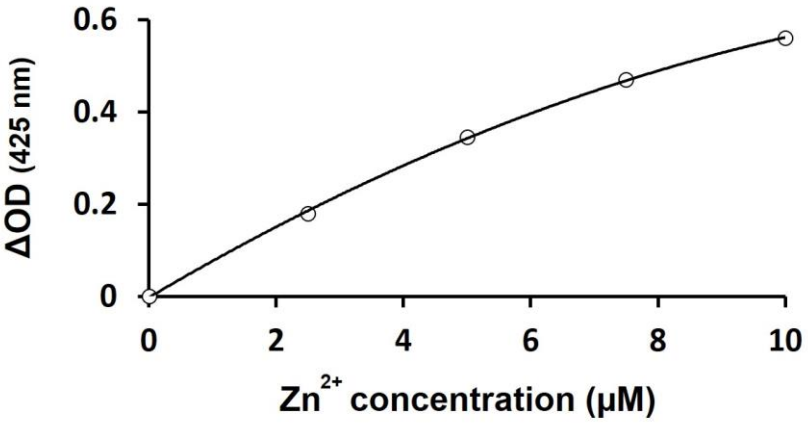
| | Standard well | Sample well | Blank well |
|---|---|---|---|
| Each diluted Standard | 50 µL | | |
| Each Sample | | 50 µL | 50 µL |
| EDTA | | | 2 µL |
| Working Reagent | 200 µL | 200 µL | 200 µL |
| Tap plate to mix briefly and thoroughly. Incubate for **30 minutes** at **room temperature**. | | | |
| Read the absorbance at **O.D. 425 nm** (420-426 nm). | | | |

## CALCULATION OF RESULTS

1. Subtract blank OD (distilled water, S5) from the standard OD values and plot the ΔOD against $Zn^{2+}$ standard concentrations. Calculate ΔOD for the Sample (= $OD_{SAMPLE}$ - $OD_{BLANK}$). Determine the sample $Zn^{2+}$ concentration from the standard curve by non-linear regression fitting with a single-site saturation binding function ($\Delta OD = a \times [Zn^{2+}] / (b + [Zn^{2+}])$.
2. If the $Zn^{2+}$ concentration is higher than 10 µM, dilute sample in distilled water. Repeat the assay and multiply the results by the dilution factor.
3. Conversions: 1 µM zinc equals 6.5 µg/dL or 0.065 ppm (65 ppb).

## EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Zinc Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.




## QUALITY ASSURANCE

### Sensitivity

0.78 µg/dL (0.12 µM)