Technical Bulletin

Zinc Assay Kit

Catalogue number MAK496

Product Description

Zinc is an essential trace element and plays many key roles in metabolism. It is required for the activity of more than 300 enzymes, the structure of many proteins, and control of genetic expression. Zinc status affects basic processes of cell division, growth, differentiation, development, performance and aging through its requirement for synthesis and repair of DNA, RNA and protein. The common causes of zinc deficiency are low dietary intakes and low bioavailability. Clinical signs of zinc deficiency include acrodermatitis, low immunity, diarrhea, poor healing, stunting, hypogonadism, fetal growth failure, teratology, and abortion. Zinc deficiency has now been recognized to be associated with many diseases such as malabsorption syndrome, chronic liver disease, chronic renal disease, sickle cell disease, diabetes, malignancy, and other chronic illnesses.

Simple, direct, and automation-ready procedures for measuring zinc concentration in biological samples are highly desirable in Research and Drug Discovery. The Zinc Assay Kit 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 425 nm, is directly proportional to the zinc concentration in the sample.

The linear detection range of the kit is 0.12 µM (0.78 µg/dL) to 10 µM (65 µg/dL) zinc. The kit is suitable for zinc concentration determination in serum, plasma (no EDTA), urine, saliva, wastewater, soil as well as studying the effects of drugs on zinc metabolism.

Components

The kit is sufficient for 250 colorimetric assays in 96-well plates.

- Reagent A — 50 mL
  Catalogue Number MAK496A
- Reagent B — 1 mL
  Catalogue Number MAK496B
- Reagent C — 1 mL
  Catalogue Number MAK496C
- EDTA (100 mM) — 1 mL
  Catalogue Number MAK496D
- Zinc Standard (50 µM) — 1 mL
  Catalogue Number MAK496E

Equipment Required but Not Provided

- Pipetting devices and accessories.
- Spectrophotometer (OD 425 nm) and cuvettes for procedure using cuvette.
- Spectrophotometric multiwell plate reader.
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes.

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components Reagent B and Reagent C at -20 °C and other components at 4 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate to room temperature prior use. Reagents B and C: Vortex before assay.

Procedure

All Samples and Standards should be run in duplicate.

Sample Preparation

Note: Zn^{2+} chelators (e.g., EDTA, EGTA) should be avoided during Sample preparation. Physiological concentrations of other metal ions do not interfere.

Serum and plasma Samples should be clear and free of turbidity or precipitates. If present, precipitates should be removed by filtration or centrifugation on a table centrifuge. Prior to assay, dilute serum, or plasma samples 5-fold (Dilution Factor (DF) = 5) in purified water.

Colorimetric Standard Curve Preparation:

1. Using the supplied 50 µM Zinc Standard (MAK496E) and purified water, prepare Standards in 1.5 mL microcentrifuge tubes as described in Table 1.

Table 1.
Preparation of Zinc Colorimetric Standards

Well	50 µM Standard	Purified Water	Zn2+ (µM)
1	20 µL	80 µL	10.0
2	15 µL	85 µL	7.5
3	10 µL	90 µL	5.0
4	5 µL	95 µL	2.5
5	0 µL	100 µL	0

Working Reagent

Mix enough reagent for the number of assays to be performed. If using 96-well plate procedure, prepare 208 µL of Working Reagent for each well, according to Table 2. If using cuvette procedure, prepare 832 µL of Working Reagent for each cuvette, according to Table 2.

Table 2.
Preparation of working reagent

Reagent	Working Reagent (well)	Working Reagent (cuvette)
Reagent A	200 µL	800 µL
Reagent B	4 µL	16 µL
Reagent C	4 µL	16 µL

Measurement

Note: Because the shift in the peak wavelength (from 413 nm to 425 nm) is very small, the color change is not visually evident.

Using 96-well plate:

1. Transfer 50 µL of the Zinc Colorimetric Standards into separate wells of a clear, flat-bottom 96-well plate.
2. Transfer 50 µL Sample and Sample Blank (50 µL Sample + 2 µL EDTA) into wells of a clear flat-bottom 96-well plate.
3. Add 200 µL working reagent to all wells and tap plate lightly to mix.
4. Incubate for 30 minutes at room temperature and read optical density (OD) at 425 nm (range 420 - 426 nm).

Using cuvette:

1. Transfer 200 µL Standards to appropriately labelled tubes.
2. Transfer 200 µL Sample (for Sample Blank: 200 µL Sample + 8 µL EDTA) to separate, respective tubes.
3. Add 800 µL working reagent and tap lightly to mix.
4. Incubate for 30 minutes and read optical density at 425 nm.

Results

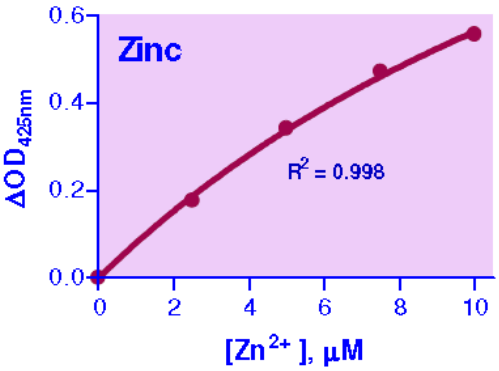
1. Subtract the OD value of Standard #5 (Blank) from the remaining Standard OD values.
2. Plot the ΔOD against Zn^{2+} standard concentrations and determine the slope.
3. Calculate ΔOD for the Sample using the below equation:
   $\Delta OD_{Sample} = OD_{Sample} - OD_{SampleBlank}$
4. Determine the Sample Zn^{2+} concentration from the standard curve by non-linear regression fitting with a single-site saturation binding function using the below equation:
   $\Delta OD = a \times [Zn^{2+}] / (b + [Zn^{2+}])$.

Note: If the Zn^{2+} concentration is higher than 10 µM, dilute sample in purified water. Repeat the assay and multiply the results by the dilution factor.

5. Conversions: 1 µM zinc equals 6.5 µg/dL or 0.065 ppm (65 ppb).

Figure 1.

Typical Colorimetric Zinc Standard Curve



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