Zinc Colorimetric Assay Kit

Catalog Number: MBS841453

(100 assays; Store kit at +4°C)

I. Introduction:

Zinc, a metallic chemical element, symbol Zn and atomic number 30 is chemically similar to Magnesium due to its similar size and sole oxidation state of +2. Zinc is an essential mineral of great biological significance since many enzymes require it as an essential cofactor. Examples of zinc's biological roles include signal transduction, gene expression, regulation of apoptosis, synaptic plasticity and prostate gland function. The Zinc Assay Kit is a convenient colorimetric assay in which Zinc binds to a ligand with development of absorbance at 560nm. It can be used with biological samples such as serum, plasma, csf or urine.

II. Kit Contents:

Components	100 assays	Cap Code
Zinc Reagent 1	16 ml	WM
Zinc Reagent 2	4 ml	Amber
7% TCA	5 ml	Clear
Zinc Standard (50 mM)	0.1 ml	Yellow

III. Storage and Handling:

Store the kit at +4°C and protect from light. Read the entire protocol before performing the assay. **Synthetic rubber and glass can contain zinc which may leach into samples. For highest accuracy all glassware should be washed with dilute HCl, rinsed with distilled water and dried prior to use. Sample tubes such as Vacutainer® and similar devices should be sealed with Parafilm® rather than the butyl rubber stopper. Chelators such as EDTA will give artifactually low Zinc levels and should be avoided. Heparin, citrate and oxalate are acceptable anticoagulants. Most blood zinc (80%) is contained in erythrocytes and hemolysis will release large amounts into the serum. Abnormally high serum values obtained suggest the collection of another sample and retesting.**

IV. Reagent preparation:

The reagents are ready to use as supplied. Add 4 parts of Zinc reagent 1 to 1 part Zinc reagent 2. Make only as much Zinc reaction mix as is needed for samples and standards to be run. Each sample or standard requires 200 µl of reagent mix. Once mixed, the Zinc reaction mix is good for 2 days at room temperature or 1 week at 4°C.

V. Assay Protocol:

1. **Standard Curve Preparation:**

   Dilute the Zinc Standard to 0.5 mM by adding 10 µl of the 50 mM Zinc Standard to 990 µl of dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells. Adjust volume to 50 µl/well with dH_2O to generate 0, 1, 2, 3, 4, 5 nmol/well of the Zinc Standard.

2. **Sample Preparation:**

   Samples containing significant amounts of protein such as serum, plasma or csf should be deproteinized by adding 50 µl of the 7% TCA solution to 50 µl of the sample. Spin at top speed for 5 minutes. Add 20-50 µl of the sample(s) to a 96 well plate; bring the volume to 50 µl/well with dH_2O. Urine samples should be acidified to pH 3-4 to dissolve any sediment which can bind zinc. 1-2 drops of concentrated HCl per 15 ml should be sufficient for this purpose. Acidified urine can be used directly (20-50 µl per well).

   Notes:

   a) Some samples might need the neutralization step following the addition of TCA solution.

   b) Sample(s) can be also deproteinized using Catalog #: MBS841870.

3. **Reaction:**

   Add 200 µl of Zinc reaction mix to each standard and sample, incubate 10 minutes at room temperature.

4. **Read:**

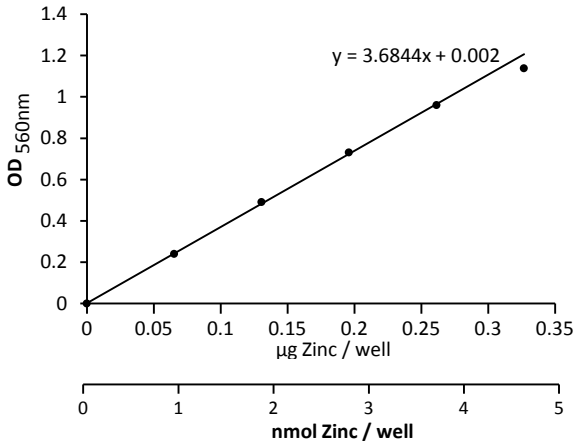
   Measure OD at 560 nm in a microplate reader.

5. **Calculation:**

   Correct background by subtracting the value derived from the 0 Zinc Standard from all readings (The background reading can be significant and must be subtracted). Plot the Zinc Standard curve. Read Zinc sample concentrations from the standard curve:

$$C = S_a/S_v \quad \text{nmol/µl or mM,}$$

Where S_a is the sample amount (in nmol) from standard curve
S_v is the sample volume (µl) added into the wells
Zinc MW: 65.384 g/mol



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