SIGMA-ALDRICH®

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Product Information

Zinc Assay Kit

Catalog Number **MAK032**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description
Zinc, one of the most abundant trace metals in living organisms, contributes to a variety of biological functions including neurological development, nucleic acid metabolism, and signal transduction. Zinc is utilized as a cofactor for many enzymes, such as carbonic anhydrase, which is important to carbon dioxide regulation, and carboxypeptidase, which cleaves peptide linkages.

The Zinc Assay kit provides a simple and direct procedure for measuring zinc in a variety of samples, including serum, plasma, urine, or cerebral spinal fluid (CSF). Zinc ions bind to a ligand, which results in a colorimetric (560 nm) product proportional to the amount of zinc present.

Components
The kit is sufficient for 100 assays in 96 well plates.

Component	Volume
Zinc Reagent 1 Catalog Number MAK032A	16 mL
Zinc Reagent 2 Catalog Number MAK032B	4 mL
7% TCA Catalog Number MAK032C	5 mL
Zinc Standard, 50 mM Catalog Number MAK032D	0.1 mL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Concentrated HCl (Catalog Number H1758)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents.
Note: 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 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.

Zinc Reagent Mix – Add 4 parts of Zinc Reagent 1 to 1 part of Zinc Reagent 2. Each sample or standard requires 200 μL of Zinc Reagent Mix. Make only as much as is needed for samples and standards to be run. Zinc Reagent Mix is stable for 2 days when stored at room temperature or 1 week at 2–8 °C.

Storage/Stability
The kit is shipped on wet ice and storage at 2–8 °C, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate. Use ultrapure water for the preparation of samples and standards.

Zinc Standards for Colorimetric Detection

Dilute 10 μL of the 50 mM Zinc Standard with 990 μL of water to prepare a 0.5 mM standard solution. Add 0, 2, 4, 6, 8, and 10 μL of the 0.5 mM standard solution into a 96 well plate, generating 0 (blank), 1, 2, 3, 4, and 5 nmole/well standards. Add water to each well to bring the volume to 50 μL.

Sample Preparation

Samples containing significant levels of protein, such as serum, plasma, or CSF, should be deproteinized by adding 50 μL of 7% TCA solution to 50 μL of sample. Mix well and centrifuge the samples at 13,000 × *g* for 5 minutes to remove insoluble material. Add 20–50 μL of supernatant to wells. Bring samples to a final volume of 50 μL with water.

Urine samples should be acidified, pH 3–4, to dissolve any sediment, which can bind zinc. The addition of 1–2 drops of concentrated HCl (not provided) per 15 mL of urine sample should be sufficient. Add 20–50 μL of acidified urine sample to wells. Bring samples to a final volume of 50 μL with water.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction

1. Add 200 μL of the Zinc Reagent Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 10 minutes at room temperature. Cover the plate and protect from light during the incubation.

2. Measure the absorbance at 560 nm (A_{560}).

Results

Calculations

The background for the assay is the value obtained for the 0 (blank) Zinc standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate Zinc standards to plot a standard curve.
Note: A new standard curve must be set up each time the assay is run.

Using the corrected measurement, the amount of zinc present in the samples may be determined from the standard curve.

Concentration of Zinc

$$S_a/S_v = C \text{ (nmole/μL, or mM)}$$

S_a = Amount of zinc in unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added to reaction well
C = Concentration of zinc in sample

Zinc atomic weight: 65.384 g/mole

Sample Calculation

Amount of zinc (S_a) = 2.84 nmole (from standard curve)
Sample volume (S_v) = 50 μL

Concentration of zinc in sample

2.84 nmole/50 μL = 0.0568 nmole/μL

0.0568 nmole/μL × 65.384 ng/nmole = 3.71 ng/μL

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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