ab102507

Zinc Quantification Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Zinc levels in various samples

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 6a, Last Updated 19 May 2023

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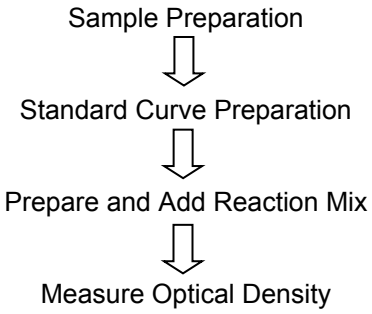
1. Overview

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, because many enzymes require it as an essential cofactor.

Examples of zinc's biological roles include signal transduction, gene expression, and regulation of apoptosis, synaptic plasticity and prostate gland function.

Abcam's Zinc Quantification Kit is a convenient colorimetric assay in which Zinc binds to a ligand that will developed and detected at an absorbance at OD_{560} nm. The assay can be used with biological samples such as serum, plasma, CSF or urine with detection sensitivity 0.2 µg/ml (~1 – 3 µM).

2. Protocol Summary



3. Materials Supplied

Item	Quantity
Zinc Reagent 1	16 mL
Zinc Reagent 2	4 mL
TCA (7%)/7% TCA	5 mL
Zinc Standard/Zinc Standard (50 mM)	100 µL

4. Storage and Stability

Upon arrival, store the kit at +4°C and protected from light.
Read the entire protocol before performing the assay.
The reagents are ready to use as supplied.

- 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 artificially low zinc levels and should be avoided in lysis buffers for all samples. For blood samples, 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 re-testing.

5. Materials Required, Not Supplied

- Distilled water or MilliQ
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker

- (Optional): HCl, for urine samples

6. Assay Protocol

1. **Sample Preparation:**
   a) **Cell lysates and tissue extracts:** harvest cells or homogenize tissue with an EDTA-free lysis buffer. Spin down to get rid of cell debris and transfer supernatant to clean tubes.

      Lysates generally contain significant amounts of protein, so they should be deproteinized by adding 50 µl of the TCA (7%)/7% TCA solution per 50 µl of the sample (add 1x volume TCA to 1x volume sample).

      Spin lysates-TCA mix at top speed for 5 minutes.

      Add 20 – 50 µl of sample to a 96-well plate; if volume of cell is < 50 µl, bring the volume to 50 µl/well with dH_2O.

   b) **Urine samples:** urine samples should be acidified to pH 3 – 4 to dissolve any sediment that could bind to zinc. Add 1 – 2 drops of concentrated HCl per 15 ml sample.

      Acidified urine can be used directly in the assay (20 – 50 µl sample/well).

   c) **Other liquid samples (Cell culture media, plasma, serum and other biological fluids):** liquid samples can be assayed directly. However, samples that contain significant amounts of protein such as serum, plasma and CSF should be deproteinized prior the assay.

Add 1x volume of the TCA (7%)/7% TCA solution to 1x volume of sample (for example, 50 µl of TCA (7%)/7% TCA to 50 µl of sample).

Spin sample-TCA mix at at top speed for 5 minutes.

Add 20-50 µl of the sample(s) to a 96 well plate; if volume of cells is < 50 µl, bring the volume to 50 µl/well with dH_2O.

For unknown samples, we suggest including several dilutions for each sample so that the reading will be within the standard curve range.

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

2. Standard Curve Preparation:

Prepare a 0.5mM Zinc Standard by adding 10 µl of the 50 mM Zinc Standard to 990 µl of dH_2O and mixing 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.

3. Reaction Mix:

Add 4x parts of Zinc Reagent 1 to 1x 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.

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

4. Measurement:

Measure assay at OD_{560nm} in a microplate reader.

7. Data Analysis

Correct background by subtracting the value derived from the zero 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:

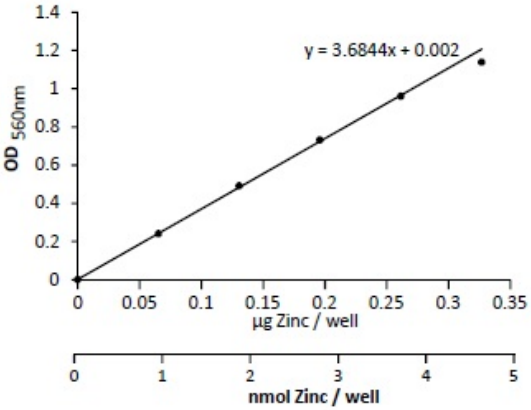
Concentration = Sa / Sv nmol/µl or mM,

Where

Sa is the sample amount (in nmol) from standard curve

Sv is the sample volume (µl) added into the wells

Zinc MW: 65.384 g/mol


y = 3.6844x + 0.002
OD 560nm
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
µg Zinc / well
0
1
2
3
4
5
nmol Zinc / well

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

For all technical or commercial enquiries please go to:

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