

**ab176725**

# **Zinc Quantification Kit (Fluorometric)**

## **Instructions for Use**

For detecting zinc concentrations in biological samples.

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

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# 1. Overview

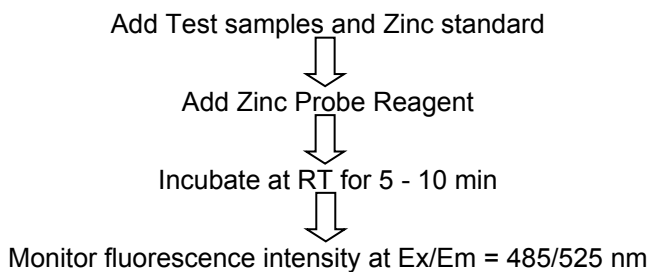
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Zinc is an essential trace mineral element that plays an important role in a number of biological processes. It is an essential factor required for many enzymes, protein structures, and control of genetic expression. Zinc status also affects basic processes of cell division, growth, differentiation, development, and aging. Clinical signs of zinc deficiency include acrodermatitis, low immunity, diarrhoea, poor healing, stunting, hypogonadism, fetal growth failure, teratology and abortion. Simple, direct and automation-ready procedures for measuring zinc ion are highly desirable in research and drug discovery.

Abcam's Zinc Quantification Kit (Fluorometric) (ab176725) provides a robust method for detecting zinc concentration in biological samples using our proprietary Zn Detector, in which Zinc binds to the probe with enhanced fluorescence at Ex/Em= 485/525 nm. The Zinc probe exhibits a large increase in fluorescence in response to  $\text{Zn}^{2+}$  (greater than 200~300 folds). It has high  $\text{Zn}^{2+}$  -specificity with little responses to other metals, including  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ , and  $\text{Cu}^{2+}$ . The assay can be used with biological samples such as serum, plasma, and urine with detection sensitivity at 0.2  $\mu\text{M}$  (13 ng/mL).

## 2. Protocol Summary

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### 3. Kit Components

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Item	Quantity	Storage upon arrival	Storage after use
Zn Detector	1 vial	-20°C	-20°C
Assay Buffer	15 mL	-20°C	-20°C
ZnCl <sub>2</sub> Standard	1 vial	-20°C	-20°C

### 4. Storage and Stability

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Upon arrival, store the kit at -20°C and protected from light. Please read the entire protocol before performing the assay. Avoid repeated freeze/thaw cycles.

Warm all buffers to room temperature before use. Briefly centrifuge all small vials prior to opening.

## 5. Materials Required, Not Supplied

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- 96 or 384-well black plate
- Multi-well fluorescence plate reader

## 6. Assay Protocol

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### 1. $\text{ZnCl}_2$ standards and test samples preparations:

- a) Add 10  $\mu\text{L}$  of 100 mM  $\text{ZnCl}_2$  Standard into 990  $\mu\text{L}$  Assay Buffer to get 1 mM  $\text{ZnCl}_2$  standard solution.
- b) Add 100  $\mu\text{L}$  of 1 mM Zinc Standard solution to 900  $\mu\text{L}$  Assay Buffer to get 100  $\mu\text{M}$   $\text{ZnCl}_2$  Standard solution.
- c) Take 300  $\mu\text{L}$  of 100  $\mu\text{M}$   $\text{ZnCl}_2$  standard solution (from Step 1b) to perform 1:3 serial dilutions to get 30, 10, 3, 1, 0.3, 0.1 and 0  $\mu\text{M}$  serially diluted  $\text{ZnCl}_2$  standards.
- d) Dilute the test sample to 5-100  $\mu\text{M}$  range with Assay Buffer.
- e) Add 50  $\mu\text{L}$  of  $\text{ZnCl}_2$  standards, diluted sample and control into each well as shown in Table 1.

### 2. Sample preparation

**Note:** The use of Heparin and EDTS will interfere with the signal.

**Note:** The pH neutralization is important for the dye as an acidic environment will decrease the signal.

**Cell lysates and tissue extracts:**

Lyse cells or homogenize tissues with an EDTA-free lysis buffer, and centrifuge to get rid of the cell debris. Deproteinize cell lysate by adding equal volume of the 7% TCA solution to the sample and spin the mixture for 5 minutes.

After sample deproteinization using TCA, samples should be neutralized. Neutralize samples in TCA using 1M Na<sub>2</sub>CO<sub>3</sub> with 1/10 to 1/5 of the sample volume. Vortex briefly to mix well. Keep samples on ice for 5 min. Samples are now deproteinized and neutralized.

**a) Urine samples:**

If required samples need to be neutralized prior the assay

**c) Other liquid samples (Cell culture media, serum and other biological fluids):**

Liquid samples can be directly used in the assay. However, samples that contain significant amounts of protein should be deproteinized and neutralized prior the assay. (Please refer to 2a) to deproteinize.)

Samples prepared should be clear and free of turbidity or precipitates. Prior to assay, samples can be diluted in Assay Buffer in 5-100μM so that the reading will be within the standard curve range.

**3. Run Zinc assay**

- a)** Add 25 μL of Zn Detector into 5 mL Assay Buffer to make Zn assay buffer. Add 50 μL Zn assay buffer to each well of ZnCl<sub>2</sub> standard, blank control, and test samples (see Step 1e Table 1) to make the total ZnCl<sub>2</sub> assay volume of 100

$\mu\text{L}/\text{well}$ . *Note: For a 384-well plate, add 25  $\mu\text{L}$  of samples and 25  $\mu\text{L}$  of assay reaction mixture into each well.*

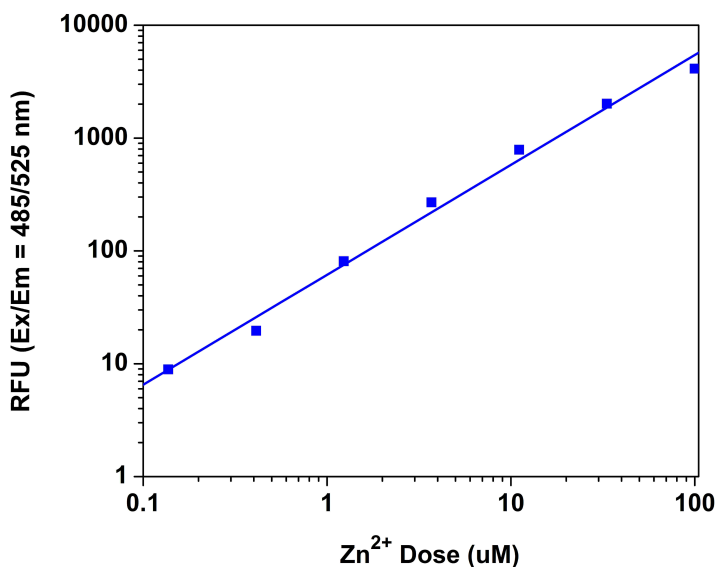
BL	BL	TS	TS	....	....	
Zn 1	Zn 1	....	....	....	....	
Zn 2	Zn 2					
Zn 3	Zn 3					
Zn 4	Zn 4					
Zn 5	Zn 5					
Zn 6	Zn 6					
Zn 7	Zn 7					

*NOTE: Zn= Zinc Standards, BL = Blank Control, TS = Test Samples.*

- b)** Incubate the reaction for 5-10 minutes at room temperature, protected from light.
- c)** Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 485/525 nm.

## 7. Data Analysis

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**Figure 1.**  $\text{ZnCl}_2$  dose response was measured on a 96-well black plate with the Abcam Zinc Quantification Kit (Fluorometric) (ab176725). As low as  $\sim 0.2 \mu\text{M}$   $\text{Zn}^{2+}$  can be detected with 5 minutes incubation time ( $n=3$ ).

## 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

Problem	Reason	Solution
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 <b>10kDa spin column (ab93349)</b>
	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)

<b>Problem</b>	<b>Reason</b>	<b>Solution</b>
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







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