Zinc Assay Kit (Fluorometric)

(Catalog # BN00673; 100 assays; Store at -20°C)

I. Introduction:

Zinc (Zn, Atomic Number: 30), is a metal with characteristics similar to Magnesium due to its size and its oxidation state of +2. This element is an essential mineral of great biological significance since many enzymes require zinc as an essential cofactor. Examples of biological roles of Zinc include signal transduction, gene expression, regulation of apoptosis, synaptic plasticity and prostate gland function. Assay Genie's Zinc Fluorometric Assay kit provides an easy and quantitative method to measure the metal ion Zinc in various biological samples. The assay is based on the ability of our proprietary probe that binds with high specificity to Zinc and becomes fluorescent. The fluorescent intensity is directly proportional to the amount of zinc, which can be quantified by measurement at Ex/Em= 435/535 nm in a microplate reader. This assay kit provides a just add-and-read, non-radioactive, and high-throughput adaptable for Zinc detection. The assay is rapid (less than 10 min) and sensitive (Limit of Detection: <0.1 µM).

II. Applications:

- Measurement of Zinc concentration in biological samples
- Analysis of the effects of drugs on Zinc metabolism

III. Sample Type:

- Biological Samples: Serum, Plasma, Urine, etc.

IV. Kit Contents:

Components	BN00673	Cap Code	Part Number
Zinc Assay Buffer	25 ml	WM	BN00673-1
Zinc Probe (in DMSO)	0.2 ml	Red	BN00673-2
Zinc Standard (50 mM)	0.1 ml	Yellow	BN00673-3

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom for Fluorometric measurement
- Multichannel or single channel Pipettes
- Multi-well spectrophotometer (Fluorescence reader)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Warm Zinc Assay Buffer to room temperature (RT) before use. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **7% TCA:** Store at 4°C. Bring to room temperature (RT) before use.
- **Zinc Probe (in DMSO) and Zinc Standard (50 mM)**: Store at 4°C or -20°C. Bring to room temperature (RT) before use. For long-term storage, aliquot, and store at -20°C. Avoid freeze/thaw.

VII. Zinc Fluorometric Assay Protocol:

1. **Sample Preparation:** Samples containing significant amounts of protein should be filtered using a 10 kD molecular weight cut off spin columns or deproteinized prior to assay: Add 50 µl of the 7% TCA solution to 50 µl of the sample, and mix well followed by centrifugation at 10,000 x g for 5 min, collect the supernatant and adjust pH to 7.2 with 6N NaOH solution (not provided). Add 10-50 µl samples into a 96-well white plate. Adjust final volume to 50 µl with Zinc Assay Buffer.

   Notes:

   Zinc concentration can vary over a wide range. Normal ranges in humans are 0.66 – 1.10 µg/ml for serum, and 300-600 µg/ml·day for urine samples. For unknown samples, we suggest to test several doses to ensure the reading are within the standard curve.

2. **Zinc Standard Curve:** Dilute the Zinc Standard to 1 mM by adding 10 µl of 50 mM Zinc Standard to 490 µl dH_2O, mix well. Dilute further to 0.02 mM (20 pmol/µl) Zinc Standard by adding 10 µl of 1 mM Zinc Standard to 490 µl dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl of the 0.02 mM Zinc Standard into a 96 well white plate to generate 0, 40, 80, 120, 160, 200 pmol/well of Zinc standard. Adjust volume to 50 µl/well with Zinc Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays (samples and standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix
Zinc Assay Buffer	49 µl
Zinc Probe	1 µl

   Add 50 µl of the Reaction Mix to each well containing the Standard and test samples. Mix well.

4. **Measurement:** Gently shake the plate for 5 min. on a shaker at room temperature protected from light. Measure the fluorescence using a microtiter plate reader at Ex/Em= 435/535 nm (no Cut-off).

5. Calculation: Subtract the 0 standard reading from all standard and sample readings. Plot the Zinc Standard Curve. Determine Zinc amount (Sa) based on the Zinc Standard Curve. The Zinc concentrations in the sample can be calculated as follow:.

C = Sa/Sv X D = pmol/µl = µM

Where: Sa = the amount of Zinc (pmol) from the calculation above

Sv = the sample volume added into reaction well (µl)

D = Sample Dilution Factor

Zinc Atomic Mass 65.384 g/mol

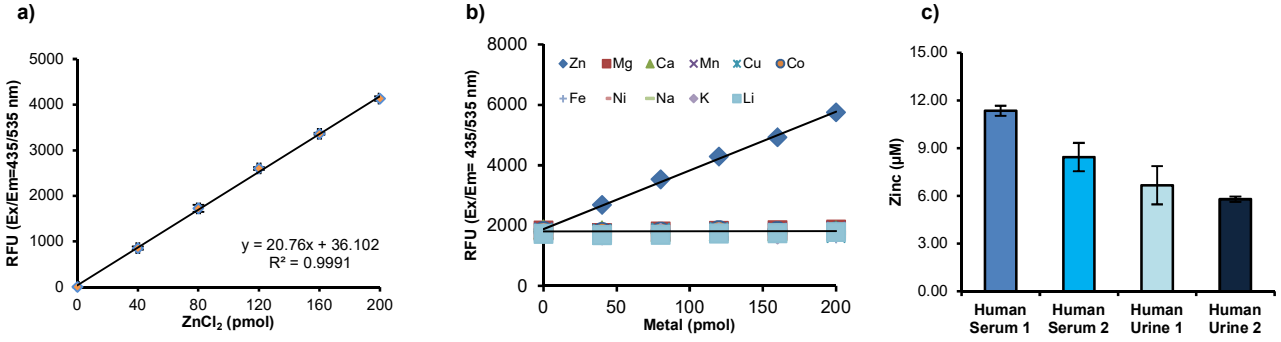

Figure: (a) Zinc Standard Curve. (b) Assay Specificity: Zinc and other mono, di and trivalent metals ions were tested to evaluate possible interferences. Interferences were found to be less than 1% when data was normalized using Zinc as 100% activity. (C) Measurement of Zinc in two Human Normal Pooled Serum samples (20 µl samples at 1:40 dilution) and two Human Normal Pooled Urine Samples (20 µl samples at 1:40 dilution). Assays were performed according to the kit protocol.

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