PRODUCT INFORMATION

Zinc Assay (5-Br-PAPS Chromogenic Method)

Cat. No. KT-760 / KT-761

INTENDED USE

The Zinc Assay (5-Br-PAPS Chromogenic Method) is a direct colorimetric assay for the quantitative determination of zinc in biological samples that does not require sample deproteinization. For research use only. Not for use in diagnostic procedures in the U.S.

PRINCIPLE

Zinc reacts with the 5-Br-PAPS chromogen in a buffered alkaline solution and forms a chelate. The intensity of this colored complex is proportional to the zinc concentration in the sample and is measured at 560 nm.

COMPONENTS

	50 tests	100 tests
	KT-760	KT-761
1. Buffer, 12 mL	x1	x2
2. Chromogen (5-Br-PAPS), 0.27 mL	x1	x2
3. Zinc Calibrator (200 µg/dL), 1.65 mL	x1	x2

Store all kit components at 4°C.

PRECAUTIONS

1. Fluctuating incubation temperature may result in variable results.
2. Use disposable test tubes and glassware washed with 1M HNO_3 or 1M HCl solution and distilled water.
3. Sample and reagent pipetting accuracy may affect assay performance. Please note that samples, calibrator, and Color Developer Solution must be dispensed accurately at the µL level.
4. The temperature of the reaction may affect the O.D. reading. Please extend or shorten the chromogen reaction time depending on the ambient room temperature if necessary.
5. For cell lysates or the tissue extraction samples, a high concentration of proteins or lipids may affect the assay result. For best results, remove proteins or lipids by ultrafiltration or centrifugation.
6. Species of zinc-porphyrins cannot be measured by this assay kit.
7. Gloves, caps, and rubber labware may cause contamination. Be sure to use clean labware.

SAMPLE PREPARATION

1. Serum or plasma

Insoluble substances in serum and plasma samples should be removed by filtration or centrifugation. EDTA-plasma samples cannot be used as EDTA interferes with this assay.

2. Tissue extracts, cell lysates, and other samples such as urine or other biological fluids:

If the sample is turbid, centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use for the assay.

If necessary, add small amounts of 6M HCl to the sample and adjust pH to 2.0 - 3.0. For example, add ~5-10 µL of 6M HCl per 1 mL of sample.

3. Tissue samples

Add 5% TCA solution, vortex 1 min. and incubate at 4 - 8°C for 30 min. Centrifuge at 6,000 rpm for 15 min. Collect the supernatant and use for the assay.

Sample pH should be between pH 2.0 to pH 8.0.

REAGENT PREPARATION

1. Prepare enough Color Developer Solution for your experiment:

Color Developer Solution		
	1 test	50 tests
Buffer	230 µL	11.5 mL
Chromogen	5 µL	250 µL

Color Developer Solution should be stored at 4℃ in the dark and used within one month after preparation. The Zinc Calibrator is ready to use.

2. Bring all reagents to room temperature before use.

ASSAY PROTOCOL (Microplate and Microplate Reader)

(Total reaction volume = 242 µL)

1. Add 230 µL of prepared Color Developer Solution to each well.
2. Add 12 µL of Blank (purified water), Zinc Calibrator, or Sample to each well.
3. Mix and incubate at room temperature for 5 minutes. Mix carefully using a pipette and avoid foaming. If a plate mixer is used for mixing, there is a risk of obtaining poor reproducibility.
4. Read the OD absorbance at 560 nm (main) and 700 nm (sub).

Sensitivity: 560 nm (maximum), 570 nm (60%), 580 nm (≤20%)

Assay Protocol				
Step	(µL)	Blank	Calibrator	Sample
1	Color Developer Solution	230	230	230
2	Purified water	12	-	-
	Zinc Calibrator	-	12	-
	Sample	-	-	12
3	Mix and incubate for 5 minutes at room temperature.			
4	Read the OD absorbance at 560 nm (main) and 700 nm (sub).			

CALCULATION OF SAMPLE CONCENTRATION

$$\frac{(OD_{560}\text{ sample} - OD_{700}\text{ sample}) - (OD_{560}\text{ blank} - OD_{700}\text{ blank})}{(OD_{560}\text{ calib.} - OD_{700}\text{ calib.}) - (OD_{560}\text{ blank} - OD_{700}\text{ blank})} \times 200 = \text{Zinc (µg/dL)}$$

Unit Conversion:
Zinc (µg/dL) x 0.153 = Zinc (µM)

Assay Example

	OD (560 nm)	OD (700 nm)	OD	ΔOD	Zinc (µg/dL)
Blank	0.062	0.030	0.032	-	-
Calibrator	0.206	0.031	0.175	0.143	-
Sample	0.117	0.033	0.084	0.052	72.7

When assaying diluted samples, multiply the result by the dilution factor.

PERFORMANCE

Assay Range: 4 - 1,000 µg/dL

Precision: Precision was evaluated using commercially available quality control serum.

Within Run Precision	Mean (µg/dL)	S.D.	C.V.%
Level 1	69.0	3.1	4.4
Level 2	109.7	2.5	2.2

Interference:

Conjugated bilirubin	No interference up to at least 15 mg/dL
Unconjugated bilirubin	No interference up to at least 15 mg/dL
Triglycerides	No interference up to at least 500 mg/dL

Shelf life: Until expiration date at 4℃. After opening any of the kit components, store at 4℃ and use within one month. **Do not freeze.**

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KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com