PRODUCT INFORMATION & MANUAL

Zinc Assay Kit (Colorimetric)

NBP3-24548

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

Zinc (Zn) Colorimetric Assay Kit

Catalog No: NBP3-24548

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.418 μmol/L

Detection range: 0.748 -46.2 μmol/L

Average intra-assay CV (%): 2.7

Average inter-assay CV (%): 4.0

Average recovery rate (%): 104

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure zinc (Zn) content in serum, plasma, urine, milk sample.

▲Background

Zinc is an essential trace element for humans, animals, plants and microorganisms. It is essential for many physiological processes, such as growth and development, lipid metabolism, immune function and so on.
Zinc deficiency may seriously affect the homeostasis of organism, which is associated with Parkinson's disease, hepatitis and cirrhosis, acrodermatitis enteropathica, diabetes and other diseases. Excessive zinc have toxic effects on cells, which can lead to apoptosis.

▲Detection principle

The zinc ion in the sample react with 5-Br-PADAP to produce the colored complex. The depth of color is directly proportional to the concentration of zinc ion. Zinc ion content can be calculated by measuring the OD values at 560 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	1.54 mmol/L Zinc Standard	0.5 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Protein Precipitator	15 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Chromogenic Agent	0.26 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 4	Buffer Solution	26 mL × 1 vial	2-8℃ , 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Microplate reader (545-575 nm), Micropipettor, Vortex mixer

Reagents

Deionized water

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. The supernatant after centrifugation must be clarified in in pretreatment step. Otherwise take the turbid supernatant to another centrifuge tube and centrifuge again.
2. As the concentration of Zn^{2+} in serum is very low, avoid zinc contamination of vessels and reagents used in the experiment.
3. Prevent the formulation of bubbles when the supernatant is transferred into the microplate.
4. The sample needs to be diluted with deionized water before determination once the concentration is beyond the linear range. The result should be multiplied by the dilution factor.

Pre-assay preparation

▲Reagent preparation

Preparation of chromogenic agent working solution:
Mix the reagent 3 and reagent 4 at a ratio of 1: 99. Prepare the fresh solution before use.

▲Sample preparation

The samples should be prepared as conventional methods. Also please refer to appendix II.

Sample requirements

1. Do not use EDTA, citrate and other metal chelators as anticoagulants.
2. Do not use hemolytic samples.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.748 -46.2 μmol/L).

The recommended dilution factor for different samples is as follows (for reference only)

Sample type	Dilution factor
Human urine	1
Human serum	1
Human milk	1
Rat serum	1

Note: The diluent is deionized water .

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A -H, standard wells; S1-S80, sample wells.

▲Detailed operating steps

1. The preparation of standard curve

Dilute 1.54 mmol/L zinc standard with deionized water to a serial concentration. The recommended dilution gradient is as follows: 0, 3.85, 7.7, 11.55, 15.4, 23.1, 30.8, 46.2 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	1.54 mmol/L Standard (μL)	Deionized water (μL)
A	0	0	1000
B	3.85	2.5	997.5
C	7.70	5	995
D	11.55	7.5	992.5
E	15.40	10	990
F	23.10	15	985
G	30.80	20	980
H	46.20	30	970

2. The measurement of samples

(1) The pretreatment of sample

Mix the sample and reagent 2 at a ratio of 1:1 and centrifuge at 13780 g for 10 min at 4℃ , then take the supernatant for detection.

(2) Standard wells: Add 0.05 mL of standard solution with different concentrations.

Sample wells: Add 0.05 mL of pretreated supernatant of sample in Step 1.

(3) Add 0.2 mL of chromogenic agent working solution to each well of Step 2.

(4) Mix fully with microplate reader for 30 s and stand for 5 min at room temperature.

(5) Measure the OD value at 560 nm with microplate reader.

▲Summary operation table

	Standard well	Sample well
Zinc standard solution with different concentrations (mL)	0.05	
Pretreated supernatant of sample in Step (1) (mL)		0.05
Chromogenic agent working solution (mL)	0.2	0.2
Mix fully and stand for 5 min at room temperature. Measure the OD value at 560 nm.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor. The standard curve is y= ax + b.

$$\text{Zn content } (\mu\text{mol/L}) = (\Delta A_{560}\text{-b}) \div a \times 2^{*} \times f$$

Note:

y: The absolute OD value of standard;

x: The concentration of standard;

a: The slope of standard curve;

b: The intercept of standard curve.

ΔA_{560}: $OD_{Sample} - OD_{Blank}$

2^{*}: Dilution factor of sample in pretreatment step.

f: Dilution factor of sample before test.

Appendix I Data

▲Example analysis

Take 0.1 mL of human serum, add 0.1 mL of reagent 2, then mix fully, centrifuge at 13780 g for 10 min at 4℃ , take the supernatant and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 0.0152 x + 0.0023, the average OD value of the sample is 0.217, the average OD value of the blank is 0.105, and the calculation result is:

Zn content (μmol/L)= (0.217-0.105-0.0023) ÷0.0152×2= 14.43 μmol/L

Appendix II Sample preparation

The following sample pretreatment methods are for reference only.

▲Serum

Collect fresh blood and stand at 25℃ for 30 min to clot the blood. Then centrifuge at 2000 g for 15 min at 4℃ . Take the serum (which is the upper light yellow clarified liquid layer) to preserve it on ice for detection. If not detected on the same day, the serum can be stored at -80℃ for a month.

▲Plasma

Take fresh blood into the tube which has anticoagulant (heparin is recommended), centrifuge at 700-1000 g for 10 min at 4℃ . Take the plasma (which is the upper light yellow clarified liquid layer, don't take white blood cells and platelets in the middle layer) to preserve it on ice for detection. If not detected on the same day, the plasma can be stored at -80℃ for a month.

▲Urine

Collect fresh urine and centrifuge at 10000 g for 15 min at 4℃ . Take the supernatant to preserve it on ice for detection. If not detected on the same day, the urine can be stored at -80℃ for a month.

▲ Milk

Collect the fresh milk sample and centrifuge at 10000 g for 10 min at 4℃ , then take the middle layer clear liquid and preserve it on ice for detection. If not detected on the same day, the sample can be stored at -80℃ for a month.

Appendix III References

1. Hafeez B, Khanif Y M, Saleem M. Role of zinc in plant nutrition - a review[J]. American Journal of Experimental Agriculture, 2013, 3(2): 374-391.
2. Salgueiro M J, Zubillaga M, Lysionek A, et al. Zinc as an essential micronutrient: A review[J]. Nutrition Research, 2000, 20(5): 737-755.
3. Ackland M L, Michalczyk A. Zinc deficiency and its inherited disorders -a review[J]. Genes & Nutrition, 2006, 1(1): 41-49.
4. Kaur K, Gupta R, Saraf S A, et al. Zinc: The Metal of Life[J]. Comprehensive Reviews in Food Science & Food Safety, 2014, 13(4): 358-376.