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Non-Protein Sulfhydryl Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Catalog Number: AK0438

Size:50T/24S

Components:

Extract I: Liquid 25 mL ×1. Storage at 4℃ .

Extract II: Liquid 25 mL ×1. Storage at 4℃ .

Reagent I: Liquid 50 mL ×1. Storage at 4℃ .

Reagent II: Powder ×1. Store at 4℃ and protect from light. Add 5 mL of anhydrous methanol before use. Mix thoroughly.

Standard: Powder ×1, 10 mg of cysteine, store at 4℃ . Add 1.65 mL of Extract solution to dissolve it into 50 μmol/mL standard solution before use.

Preparation of Extract solution: mix Extract I and Extract II according to the volume ratio of 1:1, prepare according to the number of samples and use up on the same day.

Product Description

The sulfhydryl group in organism mainly includes nonprotein sulfhydryl group and protein sulfhydryl group. Sulfhydryl compounds have important detoxification function in vivo. It has very important physiological significance to the self-regulation of organism.

The thiol group reacts with 5,5 '- dithio-bis-Nitrobenzoic Acid (DTNB) to form a yellow compound. It has a maximum absorption peak at 412 nm.

Reagents and Equipment Required but Not Provided.

Spectrophotometer, table centrifuge, water bath, adjustable pipette, 1 mL glass cuvette, methanol, mortar/homogenizer and distilled water.

Procedure

I. Sample processing

1. Animal and plant tissues: Take about 0.1 g of tissue, add 1 mL of Extract solution to prepare 10% homogenate. Centrifuge at 10000 g for 10 min at room temperature. Take the supernatant for test.

2. Cells: According to the ratio of the number of cells (10^4): the volume of the Extract solution (mL) is 500- 1000:1 to prepare. It is recommended to add 1 mL of Extract solution to 5 million cells. And the cells are broken by ultrasound (Power: 300W, ultrasound: 3s, interval: 7s, total time: 3 min). Centrifuge at 10000 g for 10 min at 4℃ . Take the supernatant on ice for test.

3. Serum (plasma) and culture medium: Add 1 mL of Extract solution to 0.1 mL of serum (plasma) or culture medium. Centrifuge at 10000 g for 10 min at room temperature. Take the supernatant for test.

II. Determination Procedure

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 412 nm and set the counter to zero with distilled water.

2. Diluted the 50 μmol/mL standard solution to 0.3、0.2、0.1、0.05、0.025、0.0125、0.00625、0.003125 μmol/mL standard solution with the extract solution. Prepare the solution when it will be used.

3. Operation table

Reagent Name (mL)	Control tube (A_C)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Supernatant	0.3	0.3	-	-
Standard	-	-	0.3	-
Reagent I	0.65	0.65	0.65	0.65
Reagent II	-	0.1	0.1	-
Distilled water	0.1	-	-	0.4
Mix thoroughly. Stay for 10 min. Determine the absorbance at 412 nm. Record as A_C, A_T, A_S, A_B. $\Delta A_T=A_T-A_C$. $\Delta As=As-A_B$.				

III. Calculation formula

1. Standard curve

Take the concentration of each standard solution as x-axis, and the corresponding ΔA standard is y-axis. Then the linear regression equation y＝kx+b is obtained. Bring ΔA into the equation to get x (μmol/mL).

2. Calculate

1) Calculate by sample weight

Non Protein Sulfhydryl content (μmol/g fresh weight)=$x\times V_E\div W=x\div W$

2) Calculate by protein concentration

Non Protein Sulfhydryl content (μmol/mL prot)=$x\times(V_E+Vs)\div V_S=11\times x$

3) Calculate by the number of cells

Non Protein Sulfhydryl content (μmol/10^4 cell)=$x\times V_E\div 500=0.002\times x$

V_E : Extract solution volume, 1 mL;

V_S : Serum (plasma) or culture medium volume ， 0.1 mL；

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

500: The number of cells, 5 million.

Note:

1. When ΔA is more than 1.2, diluted the supernatant with extract and then determined. When ΔA is too small, it is recommended to reduce the dilution ratio or increase the sample weight for determination.

Examples:

1.Add 0. 1g mouse kidney to 1mL extract solution and grind thoroughly, take supernatant and follow the determination procedure to operate, calculate: ΔA=A(T)-A(B)=0.791-0.033=0.758, standard curve:

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y=3.4566x+0. 159, calculate x=0. 1733, according with mass of sample to calculate:Non Protein Sulfhydryl content (μmol/g mass) =x÷W=0. 1733÷0. 1=1.733 μmol/g mass.

Related Products:

AK0254/AK0253	Ceruloplasmin (CP) Assay Kit
AK0456/AK0455	Total antioxidant capacity (T-AOC) Assay Kit