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Protein Free Sulfhydryl Content Assay Kit Visible

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

Operation Equipment: Spectrophotometer

Catalog Number: AK0733-50T-24S

Size:50T/24S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Sunlong staff in time.

Reagent name	Size	Preservation Condition
Extract soluion I	Liquid 25 mL×1	2-8℃
Extract soluion II	Liquid 25 mL×1	2-8℃
Reagent I	Liquid 60 mL×1	2-8℃
Reagent II	Liquid 20 mL×1	2-8℃
Reagent III	Liquid 17 mL×1	2-8℃
Reagent IV	Liquid 2 mL×1	2-8℃
Standard	Powder ×1	2-8℃

Solution Preparation:

1. **Preparation of extract solution:** before use according to the sample volume in accordance with the extract soluion I: extract soluion II = 1mL: 1 mL for the preparation, ready to use, do not mix all at once.
2. **Reagent II:** If reagent II precipitated, reagent two can be placed in a 37 ℃ water bath heating until clarified and transparent after use.
3. **Standard:** 10 mg reduced glutathione (GSH). It was prepared to 25 μmol/mL by adding 1.3 mL of distilled water before use and can be stored at 2-8°C for 4 weeks.
4. **0.125μmol/mL standard preparation:** take 50μL of 25μmol/mL standard, add 950μL of distilled water, mix thoroughly to formulate 1.25μmol/mL standard; then take 100μL of 1.25μmol/mL standard, add 900μL of distilled water, mix thoroughly to formulate 0.125μmol/mL standard.

Product Description

Sulfhydryl groups enable proteins to form disulfide bonds, thus maintaining molecular stability and functionality. In addition, sulfhydryl groups are involved in oxidative reduction reactions and have important biological roles. Changes in the content of sulfhydryl groups in cells are closely related to the occurrence and progression of various diseases, and thus sulfhydryl groups have become an important research target in the biomedical field. This kit measures the free sulfhydryl content of proteins.

Under certain conditions, sulfhydryl group will undergo nucleophilic reaction, i.e. sulfhydryl group reacts with 5,5'-dithio-bis-nitrobenzoic acid (DTNB) to produce a yellow color compound with a

maximum absorption peak at 412 nm, from which the free sulfhydryl content of protein can be calculated.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, tabletop centrifuge, water bath, adjustable pipette, 1 mL glass cuvette, acetone (>98%, AR), mortar/homogenizer/ sonicator and distilled water.

Operation procedure:

I. Sample preparation (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

1. Tissue: according to the ratio of mass (g): volume of extract (mL) is 1:5~10 (it is recommended to weigh about 0.1g, add 1mL of extract solution) add extract solution, homogenize in ice bath and centrifuge at 4℃, 3000rpm for 10min, discard the supernatant. Add 2mL of Reagent I to the precipitate, stir well to dissolve the precipitate, and use the precipitate solution as a sample for the experiment. (Note: (1) plant leaves and other samples with high fiber content, dissolve the precipitate and centrifuge at 4℃ and 3000rpm for 3min, then take the supernatant as the sample for the experiment; (2) a lot of air bubbles will be generated after adding Reagent I, please add slowly, and it is recommended to use 5mL EP tubes.)

2. Bacteria/cells: according to the ratio of the number of bacteria/cells (10^6): the volume of extract solution (mL) is 5~10:1 (it is recommended that 5 million bacteria/cells added to 1mL of the extract solution), ultrasonic crushing in an ice bath (power of 200W, ultrasound for 3 seconds, an interval of 10 seconds, a total of 3min), centrifuged at 4℃, 3,000 rpm for 10min, and discarded the supernatant. Add 2mL of Reagent I to the precipitate, stir well to dissolve the precipitate, and use the precipitate solution as a sample for the experiment. (Note: (1) If the precipitate is not completely dissolved, centrifuge at 4℃ and 3000rpm for 3min, and take the supernatant as sample for experiment; (2) a lot of air bubbles will be generated after adding Reagent I, please add it slowly, and it is recommended to use a 5mL EP tube).

3. Serum/plasma, milk and other liquids: Take 100μL of liquid sample and add 0.9mL of acetone, centrifuge at 4℃, 3000rpm for 10min, discard the supernatant. Add 2mL of Reagent I to the precipitate, stir well to dissolve the precipitate, and the precipitate dissolved solution is used as the sample for the experiment. (Note: If the measured value is small, you can change the ratio of sample to acetone, such as taking 0.2mL liquid sample and adding 0.8mL acetone or 0.3mL liquid sample and adding 0.7mL acetone, pay attention to synchronous modification of the calculation formula).

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. Operation table (recommended for operation in 5mL EP tubes)

Reagent Name (mL)	Control Tube (A_C)	Test Tube (A_T)	Blank Tube (A_B)	Standard Tube (A_S)
Sample	0.5	0.5	-	-
Distilled Water	-	-	0.5	-
Standard	-	-	-	0.5
Extract solution	0.3	0.3	0.3	0.3

Please add the extract soluion **slowly** and mix well, and blow repeatedly with a pipette tip until bubbles are no longer produced (a lot of bubbles will be produced in the meantime, open the lid and leave it for a while)			-	-
Reagent II	0.3	0.3	0.3	0.3
The supernatant was centrifuged at 3,000 rpm for 10 min at 4°C and then placed in a 1.5 mL EP tube.			Directly take a fixed volume mixture without centrifugation, and add the following reagents.	
Supernatant	0.7	0.7	0.7	0.7
Reagent III	0.3	0.25	0.25	0.25
Reagent IV	-	0.05	0.05	0.05
Mix well and let it stand at room temperature for 10 min, then determine the absorbance at 412 nm and record it as A_C, A_T, A_B and A_S, respectively. Calculate $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. The blank and standard tubes should be measured only 1-2 times. A control tube is required for each assay tube.				

III. Calculation

1. Calculate by protein concentration

   Protein Free Sulfhydryl content (μmol/mL prot) $=\Delta A_T \div (\Delta A_S \div Cs) \times Vs \div (Vs \times Cpr) \times F$

   $=0.125 \times \Delta A_T \div \Delta A_S \div Cpr \times F$

2. Calculate by sample mass

   Protein Free Sulfhydryl content (μmol/g mass) $=\Delta A_T \div (\Delta A_S \div Cs) \times Vr \div W \times F$

   $=0.25 \times \Delta A_T \div \Delta A_S \div W \times F$

3. Calculate by the Liquid volume

   Protein Free Sulfhydryl content (μmol/mL)$=\Delta A_T \div (\Delta A_S \div Cs) \times Vr \div V_{S1} \times F = 2.5 \times \Delta A_T \div \Delta A_S \times F$

4. Calculate by the number of cells

   Protein Free Sulfhydryl content (μmol/10^6 cell)$=\Delta A_T \div (\Delta A_S \div Cs) \times Vr \div N \times F = 0.25 \times \Delta A_T \div \Delta A_S \div N \times F$

Cs: Standard Tube Concentration, 0.125μmol/mL; Vs: Volume of sample added，0.5mL; Cpr: Sample Protein Concentration, mg/mL, Protein concentration is measured separately. The BCA method is recommended; W: sample mass, g; Vr: Volume of reagent I added during extraction, 2mL; V_{S1}: Sample volume of liquid added during extraction, 0.1mL; F: dilution factor; N: Total number of cells/bacteria, count by 10^6.

Note:

1. If the ΔA of the sample is <0.01, the sample volume can be increased appropriately and then measured, paying attention to the simultaneous modification of the blank and standard tubes and the calculation formula; if the ΔA of the sample is >1.5, the precipitation solution can be diluted with reagent I and then measured, paying attention to the simultaneous modification of the dilution factor in the calculation

formula.

2. Protein concentration can be determined using the BCA method.

Experimental example:

1.Take 100μL horse serum, according to the assay procedure, with 1mL glass cuvette measured $\Delta A_T = A_T - Ac=0.110-0.021=0.089$, $\Delta A_S = A_S - A_B =0.633-0.076=0.557$, according to the sample mass calculation of the total sulfhydryl content of proteins obtained:

Protein Free Sulfhydryl content (μmol/mL) = $2.5 \times \Delta A_T \div \Delta A_S$ = 0.399 μmol/mL.

2. 0.1036g of mouse liver was taken and operated in accordance with the assay steps. $\Delta A_T = A_T - Ac =0.339-0.095=0.244$, $\Delta A_S = A_S - A_B =0.633-0.076=0.557$, and the total sulfhydryl content of protein was calculated according to the sample mass:

Protein Free Sulfhydryl content (μmol/g mass) = $0.25 \times \Delta A_T \div \Delta A_S \div W \times F$ = 1.057μmol/g mass

3, take 0.1078g of soybean powder, precipitation solution diluted 2 times with reagent I, in accordance with the measurement steps, using a 1mL glass cuvette measured $\Delta A_T = A_T - Ac=0.108-0.033= 0.075$, $\Delta A_S = A_S - A_B=0.633-0.076=0.557$, according to the mass of the sample calculation of the total sulfhydryl content of protein to get:

Protein Free Sulfhydryl content (μmol/g mass) = $0.25 \times \Delta A_T \div \Delta A_S \div W \times F$ =0.625 μmol/g mass