SunLongBiotechCo.,LTD
Tel:0086-571-56623320Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Total Protein Sulfhydryl Content Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: AK0736-100T-96S

Size:100T/96S

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 Solution I	Liquid 70 mL×1	2-8℃
Extract Solution II	Liquid 70 mL×1	2-8℃
Reagent I	Liquid 110 mL×2	2-8℃
Reagent II	Liquid 20 mL×1	2-8℃
Reagent III	Liquid 7 mL×1	2-8℃
Reagent IV	Liquid 1.2 mL×1	2-8℃
Powder I	Powder×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 Solution I: Extract Solution 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:** Containing 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

The presence of sulfhydryl allows proteins to undergo disulfide bond formation, thereby maintaining molecular stability and functionality. Sulfhydryl is also involved in redox reactions and have important biological roles. In cells, changes in sulfhydryl content are closely related to the onset and progression of a variety of diseases, and thus sulfhydryl have become an important object of study in the biomedical field. **This kit determines the sum of sulfhydryl produced by protein disulfide bond breakage and its own free sulfhydryl.**

Reducing agent will cause disulfide bond cleavage to generate sulfhydryl, sulfhydryl will undergo nucleophilic reaction, that is, sulfhydryl 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 total sulfhydryl content of proteins can be calculated.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, Micro glass cuvettes/96 well plates, tabletop centrifuge, balance, adjustable pipette, acetone (>98%, AR), mortar/homogenizer/cell ultrasonic crusher 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) add extract, 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 extraction solution (mL) is 5~10:1 (it is recommended that 5 million bacteria/cells added to 1mL of the extraction 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/microplate reader for more than 30 minutes, adjust the wavelength to 412nm and set spectrophotometer to zero with distilled water.

2. Operation table (recommended for operation in 2mL EP tubes)

Reagent Name (mL)	Test Tube (A_T)	Blank Tube (A_B)	Standard Tube (A_S)
Sample	0.3	-	-
Distilled Water	-	0.3	-
Standard	-	-	0.3
Powder I	3 mg	-	-
React for 30min with the lid open, during the period every 10min with the pipette tip blowing until the bubble is no longer produced, it is prohibited to withhold the lid.		-	-
Extract Solution	0.18	0.18	0.18
Slowly add the extract and mix well, and blow repeatedly with a pipette tip until bubbles are no longer produced (during which time a large number of bubbles will be produced and left uncapped)		-	-
Reagent II	0.18	0.18	0.18
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 of the mixture without centrifugation, and add the following reagents.	
Supernatant	0.14	0.14	0.14
Reagent III	0.05	0.05	0.05
The absorbance at 412 nm was measured and recorded as **Ac**.		-	-
Reagent IV	0.01	0.01	0.01
Mix well, let it stand at room temperature for 10 min and then determine the absorbance at 412 nm, which was recorded as $\mathbf{A_T}$, A_B and A_S, respectively. Calculate $\Delta A_T = A_T - Ac$, $\Delta A_S = A_S - A_B$. The blank and standard tubes should only be measured 1-2 times. Note: In the first step of determining the absorbance at 412 nm, all the reaction solution can be poured into a micro glass cuvettes/96 well plates for determination, after which the Reagent IV can be added directly to the cuvette mixing and then continue the determination.			

III. Calculation Formula

1. Calculate by protein concentration

Total Protein Sulfhydryl content (μmol/mg 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

Total Protein 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

Total Protein Sulfhydryl content (μmol/mL)=$\Delta A_T \div (\Delta A_S \div Cs) \times Vr \div Vsl \times F = 2.5 \times \Delta A_T \div \Delta A_S \times F$

4. Calculate by the number of cells

Total Protein 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,0.3mL; Cpr: Sample Protein Concentration, mg/mL, Protein concentration is measured separately. The BCA method is recommended.; W: sample quality, g; Vr: Volume of reagent I added during extraction,2mL; Vsl: Sample volume of liquid added during extraction,0.1mL; F: dilution factor; N: Total number of cells/bacteria, in 10^6.

Note:

1. If the ΔA_T 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_T 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.

Experimental Examples:

1. Take 100μL horse serum, according to the assay procedure, with 96 well plates measured $\Delta A_T = A_T - Ac = 0.348-0.045=0.303$, $\Delta A_S = A_S - A_B = 0.417-0.105=0.312$. The total sulfhydryl content of protein was calculated according to the liquid volume:
   Total protein sulfhydryl content (μmol/mL) = $2.5 \times \Delta A_T \div \Delta A_S = 2.428$μmol/mL.
2. 0.1036g of mouse liver was taken and operated in accordance with the assay steps. $\Delta A_T = A_T - Ac = 0.475-0.107=0.368$, $\Delta A_S = A_S - A_B = 0.417-0.105=0.312$ The total sulfhydryl content of protein was calculated according to the sample mass:
   Total protein sulfhydryl content (μmol/g mass) = $0.25 \times \Delta A_T \div \Delta A_S \div W \times F = 2.846$μmol/g mass.
3. Take 0.1078g of soybean powder, precipitation solution diluted 2 times with reagent I, in accordance with the measurement steps, using 96 well plates measured $\Delta A_T = A_T - Ac = 0.762-0.084=0.678$, $\Delta A_S = A_S - A_B = 0.417-0.105=0.312$ The total sulfhydryl content of protein was calculated according to the sample mass:
   Total protein sulfhydryl content (μmol/g mass) = $0.25 \times \Delta A_T \div \Delta A_S \div W \times F = 10.079$ μmol/g mass