Protein Disulfide Bond Content Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC5790

Size:50T/24S

Components:

Extract I: Liquid 25 mL ×1. Storage at 2-8℃.

Extract II: Liquid 25 mL ×1. Storage at 2-8℃.

Preparation of extract solution: before use according to the sample volume in accordance with the extract I: extract II = 1mL: 1 mL for the preparation, ready to use, do not mix all at once.

Reagent I: Liquid 60 mL ×1. Storage at 2-8℃.

Reagent II: Liquid 20 mL ×1. Storage at 2-8℃.If reagent II precipitated, reagent two can be placed in a 37 ℃ water bath heating until clarified and transparent after use.

Reagent III: Liquid 15 mL ×1. Storage at 2-8℃.

Reagent IV: Liquid 3 mL ×1. Storage at 2-8℃.

Powder I: Powder×1. Storage at 2-8℃.

Standard: Powder ×1, Store at 2-8℃. 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.

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

Proteins are an important class of biomolecules found in all living organisms and are the basic substances of life. Disulfide bond is a chemical bond that connects the sulfhydryl groups of two different cysteine residues in different peptide chains or the same peptide chain. Disulfide bonds have a great influence on the structure of proteins and play a role in stabilizing the spatial structure of peptide chains in protein molecules, so it is particularly important to determine the content of protein disulfide bonds.

Reducing agent will make the disulfide bond cleaved, the cleaved sulfhydryl group will undergo nucleophilic reaction, that is, the 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, according to which, we can calculate the content of the disulfide bond of the protein.

Reagents and Equipment Required but Not Provided.

Spectrophotometer, tabletop centrifuge, water bath, adjustable pipette, 1 mL glass cuvette, Acetone, mortar/homogenizer/ sonicator and distilled water.

Procedure

I. Sample Processing

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 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
Powder	-	5mg	-	-
Open the lid reaction for 30min, during the period of every 10min with a pipette tip blowing until the bubble no longer produced, prohibit the reaction of withholding the lid			-	-
extract solution	0.3	0.3	0.3	0.3

Please add the extract 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, leave the lid open)				
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/96 well plates			-	-
Supernatant	0.7	0.7	0.7	0.7
Reagent III	0.25	0.25	0.25	0.25
Mix well and determine the absorbance at 412 nm, recorded as A1c, A1t, respectively.			-	-
Reagent IV	0.05	0.05	0.05	0.05
Mix well, let it stand at room temperature for 10 min and then determine the absorbance at 412 nm, which was recorded as A2c, A2t, Ab and As, respectively. Calculate ΔAt= (A2t - A1t) - (A2c- A1 c), ΔAs= As - Ab. Blank and standard tubes should only be measured 1-2 times. A control tube is required for each measurement tube. Note: In the first step of determining the absorbance at 412nm, all the reaction solution can be poured into a 1mL glass cuvette for measurement, after which the reagent can be added directly into the cuvette after mixing and continuing the measurement.				

III. Calculation Formula

1. Calculate

1) Calculate by protein concentration

Protein Disulfide Bond Content (μmol/mL prot) $=\Delta A_T \div (\Delta A_S \div Cs) \times Vs \div (Vs \times Cpr) \div 2 \times F$

$=0.0625 \times \Delta A_T \div \Delta A_S \times Cpr \times F$

2) Calculate by sample weight

Protein Disulfide Bond Content (μmol/g weight) $=\Delta A_T \div (\Delta A_S \div Cs) \times Vr \div W \div 2 \times F$

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

3) Calculate by the Liquid volume

Protein Disulfide Bond Content (μmol/mL)$= \Delta A_T \div (\Delta A_S \div Cs) \times Vr \div Vsl \div 2 \times F = 1.25 \times \Delta A_T \div \Delta A_S \times F$

4) Calculate by the number of cells

Protein Disulfide Bond Content（μmol/10^6 cell）$=\Delta A_T \div (\Delta A_S \div Cs) \times Vr \div N \div 2 \times F$

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

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 quality，g；Vr：Volume of reagent I added during extraction，2mL；Vsl：Sample volume of liquid added during extraction，0.1mL；

2: Cleavage of a disulfide bond produces two sulfhydryl groups;F：dilution factor；N：Total number of cells/bacteria，in 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.

Examples:

1.Take 100μL horse serum, according to the assay procedure, with 1mL glass cuvette measured ΔAt= (A2t - A1t) - (A2c- A1c)=（0.557-0.110）-（0.038-0.021）=0.430，ΔAs= As - Ab =0.633-0.076=0.557，according to the sample mass calculation of the total sulfhydryl content of proteins obtained:

Protein Disulfide Bond Content (μmol/mL) =1.25×$\Delta A_T \div \Delta A_S \times F$ =0.965 μmol/mL.

2. 0.1036g of mouse liver was taken and operated in accordance with the assay steps. ΔAt= (A2t - A1t) - (A2c- A1c)=（0.727-0.111）-（0.339-0.095）=0.372，ΔAs= As - Ab =0.633-0.076=0.557， and the total sulfhydryl content of protein was calculated according to the sample mass:

Protein Disulfide Bond Content (μmol/g weight) =0.125×$\Delta A_T \div \Delta A_S \div W \times F$=0.806μmol/g weight.

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 ΔAt= (A2t - A1t) - (A2c- A1c) =（1.080-0.068）-（0.108-0.033）=0.937，ΔAs= As - Ab =0.633-0.076=0.557，according to the mass of the sample calculation of the total sulfhydryl content of protein to get:

Protein Disulfide Bond Content (μmol/g weight) = 0.125×$\Delta A_T \div \Delta A_S \div W \times F$ =3.901μmol/g weight

Related Products:

BC1370/BC1375	Total Mercapto(-SH) Content Assay Kit
BC1430/BC1435	Thiol Content Assay Kit (Non-Protein Sample)
BC5800/BC5805	Total Protein Thiol Content Assay Kit
BC5890/BC5895	Protein Free Thiol Content Assay Kit