Total Sulfhydryl Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC1375

Size: 100T/48S

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 Solarbio staff in time.

Reagent name	Size	Storage
Extract Solution	Solution 60 mL×1	2-8℃
Reagent I	Solution 20 mL×1	2-8℃
Reagent II	Solution 1 mL×1	2-8℃
Standard	Powder×1	2-8℃

Solution preparation:

Standard: Powder×1, 10 mg of Reduced glutathione (GSH). Before use, add 1.3 mL distilled water to make the concentration to 25 μmol/mL. It could be stored at 2-8℃ for two weeks.

Description:

The sulfhydryl mainly includes glutathione sulfhydryl group and protein sulfhydryl group in vivo. The former can not only repair the oxidative damage protein, but also participate in scavenging the reactive oxygen species. The latter plays an important role in maintaining the protein conformation. The content of protein sulfhydryl can be determined indirectly by measuring the content of total sulfhydryl and GSH.

Sulfhydryl react with 5,5'-dithio-bis-nitrobenzoic acid (DTNB) to form yellow compound which has max absorbance peak at 412 nm.

Technical index:

Minimum detection limit: 0.0088 μmol/mL

linear range: 0.015625-1 μmol/mL

Required but not provided:

Spectrophotometer/ microplate reader, micro glass cuvette/96 well plate, balance, desktop centrifuge, constant temperature water bath, mortar/homogenizer, and distilled water

Procedure:

I. Sample preparation:

1. Animal and plant tissues: According to the ratio of tissue mass (g) to Extract solution volume (mL) of 1:5-10 (weigh about 0.1g of tissue and add 1mL of Extract solution), homogenize 8000g in an ice bath,

centrifuge at room temperature for 10 minutes, and take the supernatant for testing.

2. Serum and culture medium: directly measured. If the solution is turbid, centrifuge and take the supernatant for measurement.

II. Determination procedure.

1. Preheat spectrophotometer or microplate reader for 30 min, adjust wavelength to 412 nm, set spectrophotometer counter to zero with distilled water.

2. Standard working solution: dilute 25 μmol/mL standard solution with distilled water to 0.5、0.25、0.125、0.0625、0.03125、0.015625 μmol/mL standard solution. Prepare when the solution will be used.

3. The standard solution dilution can refer to the following table:

Number	Pre dilution concentration (mg/mL)	Standard liquid volume (μL)	Volume of standard dilution solution (μL)	Diluted concentration (mg/mL)
1	25	20	980	0.5
2	0.5	200	200	0.25
3	0.25	200	200	0.125
4	0.125	200	200	0.0625
5	0.0625	200	200	0.03125
6	0.03125	200	200	0.015625

Note: Each standard tube in the following experiment requires 40 μL of standard solution (be careful not to directly test the absorbance of the standard solution in this step).

4. Operating table.

Reagent	Control tube (A_C)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample (μL)	40	40	-	-
Standard (mL)	-	-	40	-
Reagent I (μL)	150	150	150	150
Reagent II (μL)	-	10	10	-
H_2O (μL)	10	-	-	50
Mix thoroughly, incubate at room temperature for 10 min. Detect the absorbance of 412 nm, and record it as A_C, A_T, A_S and A_B, and calculate $\Delta A_S = A_S - A_B$, $\Delta A_T = A_T - A_C$. Each test tube should be provided with one contrast tube. Standard curve and blank tube only need to be measured once or twice.				

III. Calculation

1. Standard curve drawing

According to concentration of standard solution (x， μmol/mL) and absorbance to create the standard curve, take standard solution as X-axis, ΔA_S as Y-axis. Take ΔA_T into the equation to obtain x (μmol/mL).

2. Calculation of total sulfhydryl content

A. Calculation by Sample weight:

Total Sulfhydryl (μmol/g weight) = $x \times V_{ST} \div W = x \div W$

B. Calculation by Protein concentration:

Total Sulfhydryl (μmol/mg prot) = $x \times V_{ST} \div (Cpr \times V_{ST}) = x \div Cpr$

Calculation by the volume of Serum/ Culture medium

Total Sulfhydryl (μmol/ L) = $x \times Vs \div (Vs \times 10^{-3}) = 1000x$

V_{ST}: Extraction solution volume, 1 mL;

W: Sample weight, g;

Cpr: Sample protein concentration, mg/mL.

Vs: Sample volume, 0.04 mL

10^{-3}: Unit conversion factor, 1 mL=10^{-3} L.

Note:

If the absorbance value determined by the sample is beyond the standard curve range, the sample should be diluted or concentrated properly before determination.

Recent Product citations:

[1] Chi X, Li X, Hou X, Guo S, Hu X. Facile Bioself-Assembled Crystals in Plants Promote Photosynthesis and Salt Stress Resistance. ACS Nano. 2021 Mar 23;15(3):5165-5177. doi: 10.1021/acsnano.0c10351. Epub 2021 Feb 23. PMID: 33620211.

[2] Zhang T, Wang J, Feng J, Liu Y, Suo R, Ma Q, Sun J. Effects of ultrasonic-microwave combination treatment on the physicochemical, structure and gel properties of myofibrillar protein in Penaeus vannamei (Litopenaeus vannamei) surimi. Ultrason Sonochem. 2022 Nov;90:106218. doi: 10.1016/j.ultsonch.2022.106218. Epub 2022 Nov 4. PMID: 36356497; PMCID: PMC9650070.

[3] Zhang T, Wang J, Feng J, Liu Y, Suo R, Jin J, Wang W. Ultrasonic pretreatment improves the gelation properties of low-salt Penaeus vannamei (Litopenaeus vannamei) surimi. Ultrason Sonochem. 2022 May;86:106031. doi: 10.1016/j.ultsonch.2022.106031. Epub 2022 May 10. PMID: 35569439; PMCID: PMC9118890.

[4] Yu F, Lin L, Sun J, Pan J, Liao Y, Pan Y, Bai G, Ma L, Mao J, Hu L. Cysteine Pathogenic Variants of PMM2 Are Sensitive to Environmental Stress with Loss of Structural Stability. Oxid Med Cell Longev. 2023 Jan 25;2023:5964723. doi: 10.1155/2023/5964723. PMID: 36743691; PMCID: PMC9891822.

[5] Xu J, Sun Q, Dong X, Gao J, Wang Z, Liu S. Insight into the microorganisms, quality, and protein structure of golden pompano (Trachinotus ovatus) treated with cold plasma at different voltages. Food Chem X. 2023 May 5;18:100695. doi: 10.1016/j.fochx.2023.100695. PMID: 37234402; PMCID: PMC10206424.

Related Products:

BC1300/BC1305	Ceruloplasmin (CP) Assay Kit
BC1310/BC1315	Total antioxidant capacity (T-AOC) Assay Kit

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