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

Cat No: BC1370

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 Solarbio staff in time.

Reagent name	Size	Storage
Extract Solution	Solution 40 mL×1	2-8℃
Reagent I	Solution 55 mL×1	2-8℃
Reagent II	Solution 2.5 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: 0.0048 μmol/mL

linear range: 0.0078-0.5 μmol/mL

Required but not provided:

Spectrophotometer, 1 mL glass cuvette, centrifuge, constant temperature water bath, balance, motor/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 for 30 min, adjust wavelength to 412 nm, set 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 dilution of standard solution 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	500	500	0.25
3	0.25	500	500	0.125
4	0.125	500	500	0.0625
5	0.0625	500	500	0.03125
6	0.03125	500	500	0.015625

Note: Each standard tube in the following experiment requires 200 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 (mL)	0.2	0.2	-	-
Standard (mL)	-	-	0.2	-
Reagent I (mL)	0.75	0.75	0.75	0.75
Reagent II (mL)	-	0.05	0.05	-
H_2O (mL)	0.05	-	-	0.25
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 $\triangle A_S = A_S - A_B$, $\triangle A_T = A_T - A_C$. 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) $= x \times V_{ST} \div (Cpr \times V_{ST}) = x \div Cpr$

C. 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, 0.2 mL;

1000: 1 μmol/mL=1000 μmol/ 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. Pay attention to synchronously modifying the calculation formula.

Recent Product citations:

[1] Jia F, Yu W, Li X, Chen Y, Wang Y, Ji J. Microneedles loaded with glutathione-scavenging composites for nitric oxide enhanced photodynamic therapy of melanoma. Bioeng Transl Med. 2022 Jun 17;8(1):e10352. doi: 10.1002/btm2.10352. PMID: 36684091; PMCID: PMC9842046.

[2] Yu H, Xie J. Effect of different orthogonal double frequency ultrasonic assisted freezing on the quality of sea bass. Food Chem X. 2023 May 4;18:100704. doi: 10.1016/j.fochx.2023.100704. PMID: 37215196; PMCID: PMC10196802.

[3] Niu J, Wan X, Yu GY, Jiang S, Yi RN, Wu YP, Ouyang SH, Liang L, Kurihara H, Sun WY, Zhu XF, Zhang RH, Cao YF, He JB, Duan WJ, Li YF, He RR. Phospholipid peroxidation-driven modification of chondrogenic transcription factor mediates alkoxyl radicals-induced impairment of embryonic bone development. Redox Biol. 2022 Oct;56:102437. doi: 10.1016/j.redox.2022.102437. Epub 2022 Aug 20. PMID: 36037588; PMCID: PMC9440361.

[4] Wu Y, Deng J, Xu F, Li X, Kong L, Li C, Sheng R, Xu B. The mechanism of Leuconostoc mesenteroides subsp. IMAU:80679 in improving meat color: Myoglobin oxidation inhibition and myoglobin derivatives formation based on multi enzyme-like activities. Food Chem. 2023 Dec 1;428:136751. doi: 10.1016/j.foodchem.2023.136751. Epub 2023 Jun 28. PMID: 37453392.

[5] Zhou E, Wang W, Xue X, Wang P, Wu F, Wu L, Li Q. Hydrogen peroxide oxidation modifies the structural properties and allergenicity of the bee pollen allergen profilin. Food Chem. 2023 Nov 1;425:136495. doi: 10.1016/j.foodchem.2023.136495. Epub 2023 Jun 2. PMID: 37276665.

Related Products:

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BC1310/BC1315	Total antioxidant capacity(T-AOC) Assay Kit
BC1360/BC1365	Uric acid（UA）Assay Kit

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