Total Sulfhydryl Assay Kit

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

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: NA0521

Size: 100T/48S

Components:

Extract solution: Liquid 60 mL×1, store at 4℃.

Reagent I: Liquid 20 mL×1, store at 4℃.

Reagent II: Liquid 1 mL×1, store at 4℃ and avoid light.

Standard: Powder×1, 10 mg of GSH. Add 1.3 mL distilled water to make the concentration to 25 μmol/mL. before use. store at 4℃.

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.

Required but not provided:

Scale, mortar, constant temperature water bath, spectrophotometer/microplate reader, motor/homogenizer, micro glass cuvette/96 well flat-bottom plate and distilled water.

Protocol:

I. Sample preparation:

1. Animal or plant tissue: Add 1 mL of Extract solution to 0.1 g of tissue, prepare as 10% homogenate, centrifuge at 8000 g and room temperature for 10 min. Supernatant is for test.

2. Serum/Culture medium: Detect directly.

II. Determination procedure.

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

2. Dilute 25 μmol/mL standard solution with distilled water to 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 μmol/mL standard solution. Prepare when the solution will be used.

3. Operating table.

	Control tube (A_C)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample (μL)	40	40		

Standard (mL)			40	40
Reagent I (μL)	150	150	150	150
Reagent II (μL)		10	10	
H_2O (μL)	10			10
Mix thoroughly, incubate at room temperature for 10 min, set zero with double distilled water, micro glass cuvette/ 96 well flat-bottom plate, detect 412 nm absorbance. Record as A_C, A_T, A_S, A_B. Calculate $\Delta A_S = A_S - A_B$、$\Delta A_T = A_T - A_C$				

III. Calculation

1. Using the standard solution concentration as the x-axis and ΔA_S as the y-axis, draw a standard curve to obtain the standard equation y = kx + b. Substitute the ΔA_T measurement into the formula 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/prot) = $x \times V_{ST} \div (Cpr \times V_{ST}) = x \div Cpr$

3. 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

1000: Unit conversion factor, 1 L=1000 mL.

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] Yang X, Xu J, Fu C, et al. The cataract-related S39C variant increases γS-crystallin sensitivity to environmental stress by destroying the intermolecular disulfide cross-links[J]. Biochemical and Biophysical Research Communications, 2020.

Related Products:

NA0769/NA0528 Ceruloplasmin(CP) Assay Kit

NA0768/NA0527 Total antioxidant capacity(T-AOC) Assay Kit

NA0763/NA0522 Uric acid（UA）Assay Kit