Instruction manual

* For research use only
* Store at 4–8℃

Thiol detection Assay kit
(Ellman's method)

Description

In living organisms, thiol groups exist as free cysteine, glutathione, cysteine residues in proteins (such as albumin). Since thiols, that contain thiol groups attached to a carbon atom, have a strong reducing ability, it is thought that they have important roles in the antioxidant capacity to scavenge reactive oxygen species (ROS). An imbalance between ROS production and antioxidant capacity can cause oxidative stress, that is thought to be involved in the development of cancer, Parkinson's disease, atherosclerosis, heart failure etc.

Serum protein thiol levels are thought to be an indicator of the *in vivo* reduction/oxidation (redox) status in organisms. It has been reported that patients of various disease exhibit low level of serum protein thiols. Furthermore, the oxidations of protein thiol groups contribute to the tertiary or quaternary structure of a protein.

The thiol detection assay kit (Ellman's method) measures total thiol groups in the sample. Ellman's reagent (DTNB: 5,5'-Dithiobis(2-nitrobenzoic acid)) reacts with thiol groups in the sample, and yields TNB^{2-} (5-Mercapto-2-nitrobenzoic acid), that has yellow color. The intensity of the color formed is proportional to the thiol groups in the sample. Thiol concentration can be calculated by measuring absorbance of TNB^{2-} at wavelength 412nm (380 nm to 440 nm).

Kit contents

100 tests (Catalog # : TH01DE)

R-A	Buffer solution	●	20 mL×1
R-B	Chromogen(DTNB) solution	●	3 mL×1
Standard (STD)	Calibrator N-acetyl-L-cysteine powder (as 1.0 mM Thiol)	●	Vial×1

Note

A) Use disposable test tube and glassware washed with 1 M HNO_3 or 1 M HCl solution and distilled water.

B) In the cell lysate or the tissue extract use as specimen, high concentration of proteins or lipid, may affect observed value. Please remove its by ultrafiltration or centrifugation.

C) Accuracy in pipetting volume for samples and reagents may affect the quality of assay. Please note that samples, standards and Working Reagent must be poured accurately µL level.

D) Temperature for chromogen reaction may affect optical density. Please try to extend or shorten chromogen reaction time depending on room temperature.

E) Sample must be assayed fresh. If you stored the samples, please keep in -20 ℃.

F) Repeated freeze/thawing causes damage to sample.

Required but not provided:

- Microplate reader (Wavelength 380-440 nm)
- Micro titer plate (96 wells)
- Pipette (20, 30, 200 µL)
- Pipette chips
- Distilled water

Operation

1. Sample preparation

◇Serum or Plasma

Insoluble solid in serum or plasma samples should be removed by filtration or centrifugation. EDTA-plasma is best suited.

2. Assay preparation

- R-A, R-B
  Bring all reagents under room temperature before use.

- Reconstitute of STD
  Add distilled water to STD vial to dissolve N-acetyl-L-cysteine powder. (The volume of distilled water to add is indicated on the label of the vial.)

3. Assay procedure

Procedure using microplate reader.
(250 µL per 1 assay sample)

○Assay

(1) Add 20 µL of distilled water (Blank) / STD / sample into each well.
(2) Add 200 µL of R-A to each well.
(3) Read the absorbance at 412 nm. → OD1
(4) Add 30 µL of R-B to each well and incubate at room temperature for 10 min.
(5) Read the absorbance at 412 nm. → OD2
* Select the filter: 380-440 nm at 412 nm.

Add (µL)		Assay Sample Blank OD_{Bl}	Standard OD_{Std}	Sample OD_S
1	Distilled water	20		-
	STD	-	20	-
	Assay sample	-	-	20
2	R-A	200	200	200
	↓ Mix and read the absorbance at 412 nm. (OD1)			
3	R-B	30	30	30
	↓ Mix and incubate for 10 minutes at room temperature. Read the absorbance at 412 nm. (OD2)			

○Calculations

$\Delta OD_{Std} = (OD2_{Std} - OD1_{Std}) - (OD2_{Bl} - OD1_{Bl})$
$\Delta OD_S = (OD2_S - OD1_S) - (OD2_{Bl} - OD1_{Bl})$
Thiol level (mM)= $\Delta OD_S / \Delta OD_{Std}$ X 1.0
*When a sample was diluted, please multiply the result by dilution-factor.

(Assay example)

	OD1 (412 nm)	OD2 (412 nm)	OD	ΔOD	Thiol (mM)
Blank	0.029	0.041	0.012	-	-
Standard	0.031	0.302	0.271	0.259	-
Sample	0.070	0.174	0.104	0.092	0.355

Performance

Measuring range 0.05 – 2.0 mM
Imprecision Imprecision was evaluated using serum.

Within run

	Mean mM	S.D	C.V %
Level 1	0.17	0.01	1.52
Level 2	0.26	0.01	3.47

Run to run

	Mean mM	S.D	C.V %
Level 1	0.21	0.00	1.31
Level 2	0.27	0.00	1.81

Expiration date and preservation conditions

Storage conditions: Store at 2-8°C. Don't freeze.
Expiration: Use before 12 months from date of manufacture.
After the bottles are opened, the kit should be used in 1 month.

Reference

1.) Ellman GL "Tissue sulfhydryl groups." *Arch. Biochem. Biophys. 82 (1): 70–7. (1959).*
2.) Hiroharu Einaga, Eiji Yoshihara, Yoshiyuki Matsuo, Junji Yodoi"Oxidative stress and redox regulation – protein oxidative modification and action-" *Journal of analytical Bio-Science, Vol.32, No4 (2009)*
3.) Banne AF1, Amiri A, Pero RW. "Reduced Level of Serum Thiols in Patients with a Diagnosis of Active Disease. "*Journal of Anti-Aging Medicine.Vol.6, Number 4, 2003*

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