Ellman Assay

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Objective

Determination of total sulfhydryl groups, protein-bound sulfhydryl groups, and free sulfhydryl groups in biological samples using DTNB (Ellman's reagent). Sensitivity 50 μM to 1000 μM.

Sedlak J, Lindsay RH (1968) Anal. Biochem. 25:192-205.

Chemicals

5,5'-Dithio-bis(2-nitrobenzoic acid) (DTNB); MW 396.3; Sigma D-8130
Ethylenediaminetetraacetate, tetrasodium salt, dihydrate (EDTA); MW 416.21; 99 %; Sigma ED4SS
Glutathione (GSH); MW 307.3; 99 %; reduced; Sigma G-4251
Methanol; p.A.; J.T. Baker 8045
Trichloroacetic acid (TCA); 72 %; unknown unknown
Triethanolamine-HCl (TEA); MW 185.7; Merck 1.08357
Trizma Base; MW 121.1; Sigma T-1503

Devices

Microplate reader
Multichannel pipettors
Repeaters
RIA-tube racks

Solutions

Stock solutions

Tris·HCl pH 8.2

24.22 g	Trizma Base	final: 30 mM
8.32 g	EDTA	final: 3 mM

Dissolve in 800 ml MilliQ. Adjust pH with 3 N HCl to 8.2. Add MilliQ to make 1000 ml. Store at room temperature.

Tris·HCl pH 8.9

48.44 g	Trizma Base	final: 262 mM
8.32 g	EDTA	final: 13 mM

Dissolve in 800 ml MilliQ. Adjust pH with 3 N HCl to 8.9. Add MilliQ to make 1000 ml. Store at room temperature.

TEA

1.86 g	TEA	20 mM

Add MilliQ to make 500 ml. Store at 4°C.

TCA

13.9 ml	TCA	10 %

Stir carefully into 70 ml MilliQ. Add MilliQ to make 100 ml. Store at room temperature.

DTNB

29.7 mg	DTNB	final: 150 μM

Dissolve in 25 ml Methanol. Store at 4°C.

Fresh solutions

Glutathione stock

31.1 mg	GSH	20 mM

Add TEA to make 5 ml. Prepare fresh daily and keep on ice until used.

Procedures

GSH standard

Add 950 μl TEA to 50 μl of Glutathione stock to make a 1 mM GSH stock. Prepare the following concentration series:

Table 1. GSH standard concentration series

Conc. (μM)	**0**	**50**	**100**	**250**	**500**	**1000**
GSH 1 mM (μl)	0	25	50	125	250	500
TEA (μl)	500	475	450	375	250	0

Total-SH

Add the following components to RIA tubes:

20 μl	Sample/Standard
75 μl	Tris·HCl pH 8.2
25 μl	DTNB
400 μl	Methanol

Spin down samples at 3000 x g for 5 min at room temperature. Using a multichannel pipettor, transfer 3 x 90 μl supernatant into a flat-bottom microplate. Measure extinction at 412 nm.

Free SH

Use a V-bottom microplate to set up the following reaction:

50 μl	Sample/Standard
2 x 25 μl	TCA

Spin down for 15 min at 1500 rpm and room temperature and keep the supernatant for later use.

Set up the following reaction in a flat-bottom microplate:

200 μl	Tris·HCl pH 8.9
20 μl	DTNB
50 μl	Supernatant from the TCA precipitation

Measure at 412 nm.

Protein-bound SH

Calculate the difference of the Total-SH and the Free SH.