BIOMEDICAL RESEARCH SERVICE CENTER
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Free Thiol Assay Kit (Cat #: A-120)

COMPONENTS:	Thiol Assay Buffer:	20 ml (for 200 assays); store at room temperature
	10x Cell Lysis Solution:	25 ml; store at 4°C
	DTNB:	0.1 g, store at RT

PRODUCT DESCRIPTION: Measurement of free thiols (sulfhydryl groups) associated with small molecules and cysteine residues on proteins provides important information regarding the redox status of the biological system. The kit is based on quantification of free thiols by Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid; DTNB), which releases 2-nitro-5-thiobenzoate (NTB) upon reaction with thiols. NTB exhibits a yellow color with an extinction coefficient of 14.15 $mM^{-1}cm^{-1}$ at 412 nm, allowing for detection of as low as 2 - 10 µM free thiols. Sample deproteination, which requires TCA precipitation, detects free thiols associated with small molecules. Reagents are stable for many years if handled properly.

SAMPLE PREPARATION:

Cell Lysate- Pellet at least ~10^6 PBS-washed cells and remove PBS completely. Store the cell pellet at -80ºC until use. Prepare enough 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Add 50 − 100 µl ice-cold 1x Cell Lysis Solution to cell pellet. Pipette cells up and down thoroughly, but avoid foaming. Leave lysate on ice for 5 min with gentle agitation. If lysate is viscous, add more 1x Cell Lysis Solution and repeat pipetting. Centrifuge lysate in a refrigerated microfuge at maximum speed (~13,000 rpm) for 3 min and harvest the supernatant for thiol assay.

Tissue Homogenate- Tissue sample should be washed with PBS thoroughly to remove blood cells. Use ~50 mg tissue per ml of 1x Cell Lysis Solution for tissue homogenization. Centrifuge homogenate in a refrigerated microfuge at maximum speed for 3 min and harvest the supernatant for thiol assay.

Protein Assay- Perform protein assay to determine cell/tissue lysate protein concentration. Normalize (equalize) sample protein concentration by diluting with ice-cold 1x Cell Lysis Solution to 0.5 − 2 mg/ml. Keep protein samples on ice at all times and use for thiol assay as soon as possible. Store samples at -80ºC after assay. The cell/tissue lysates are used to measure TOTAL free thiols associated with small molecules AND soluble proteins.

Deproteination- Mix cell/tissue lysate with an equal volume of ice-cold 10% TCA (not provided) and incubate on ice for 30 min. Spin tube 5 min in a microfuge at maximum speed. Transfer supernatant to another microtube. Add 0.5 ml Tris/EDTA-saturated Ether to the supernatant. Close tube and vortex 20 sec. Spin tube 10 sec. Remove Ether from the top layer. Repeat Ether extraction twice. Finally, keep tube uncapped in the fume hood for 30 min. Store sample at -80ºC. For protocol on TCA precipitation, please visit our webpage at http://www.bmrservice.com/SupplementTCA.html. Deproteinized samples are used to measure free thiols associated with small molecules (glutathione, free cysteine, etc).

ASSAY PROTOCOL:

Preparation of Working Assay Solution- Prepare fresh enough Working Assay Solution by dissolving 4 mg DTNB per ml of ice-cold Thiol Assay Buffer. Keep solution on ice during assay. Unused portion of Working Assay Solution can be stored at -20ºC for several weeks.

Thiol Assay- Samples upon thawing should be placed on ice. Add 20 µl sample to each well of a 96-well plate. Include a blank well containing 20 µl 1x Cell Lysis Solution or dH_2O. Reaction is initiated by adding 100 µl Working Assay Solution to each well followed by gentle agitation. Allow reaction to proceed at room temperature for 10 min. Read $O.D._{412\ nm}$ using a plate reader. Blank well reading should be subtracted from each sample well reading. Use the subtracted sample reading for calculation of thiol concentration.

Sample thiol concentration (mM) = sample $O.D._{412\ nm}$ × 6 ÷ (0.6 × 14.15) = sample $O.D._{412\ nm}$ × 0.7067

Note:

- DTT (0.1 – 1 mM) can be used as a positive control for the thiol assay.
- The sample light path in the 96-well plate is typically ~0.6 cm, which is used for calculation of thiol concentration.
- Please contact us or visit the product webpage for MSDS information on DTNB, TX-100, and EDTA.