Product Information

Thioredoxin Reductase Activity Colorimetric Assay Kit

I. Kit Contents:

Components	K2175-100 100 assays	Cap Color	Part Number
TrxR Assay Buffer	25 ml	WM	K2175-C-1
TNB Standard (lyophilized)	1 vial	Brown	K2175-C-2
DTNB (lyophilized)	1 vial	Red	K2175-C-3
NADPH (lyophilized)	1 vial	Blue	K2175-C-4
TrxR Positive Control	1 vial	Green	K2175-C-5
TrxR Inhibitor (lyophilized)	1 vial	Clear	K2175-C-6

II. Introduction:

Thioredoxin reductase (TrxR) is an ubiquitous enzyme that mediates various cellular processes (e.g. p53 activity, cell growth and defense of oxidative damage). The mammalian TrxR downregulate non-disulfide substrates such as lipoic acids, selenite and hydrogen peroxide etc. Thioredoxin Reductase Assay Kit offers a fast and easy colorimetric assay for measuring TrxR activity in different sample. In this assay, TrxR catalyzes the reduction of 5, 5'-dithiobis (2-nitrobenzoic) acid (DTNB) with NADPH to 5-thio-2-nitrobenzoic acid (TNB2-), produce an intense yellow color ((λmax = 412 nm.). Because glutathione peroxidase and glutathione reductase can also reduce DtNB in crude biological ample, TrxR specific inhibitor is used to detect the TrxR specific activity. Two assays are carried out: the first measurement is of the total DTNB reduction by the sample, and the second one is the DTNB reduction by the sample with the TrxR specific inhibitor. The DTNB reduction by TrxR is the difference between the two results.

III. Storage and Handling:

Store the kit at -20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge vials prior to opening. Read the entire protocol before performing the assay.

IV. Reagent Reconstitution and General Consideration:

TNB Standard: Dissolve lyophilized TNB standard into 0.5 ml Assay Buffer to generate 5 mM TNB Standard. The TNB standard solution is stable for 2 month at -20°C.

DTNB Solution: Dissolve DTNB into 0.9 ml Assay Buffer, sufficient for 100 assays. The DTNB solution is stable for 1 week at 4°C or 2 month at -20°C.

NADPH: Dissolve one vial with 0.22 ml dH_2O; sufficient for 100 assays. The solution is stable for 1 week at 4°C or 2 month at -20°C.

TrxR Positive Control: Reconstitute with 90 µl Assay Buffer to generate ~0.2 mU/µl TrxR; it is stable for 1 day at 4°C or 2 month at -20°C.

TrxR Inhibitor: Dissolve TrxR Inhibitor into 1.2 ml Assay Buffer, sufficient for 100 assays. The TrxR Inhibitor solution is stable for 2 month at -20°C.

Ensure that the Assay Buffer is at room temperature before use. Keep samples, NADPH, TrxR inhibitor, TrxR Positive Control on ice during the assay.

V. Thioredoxin Reductase Activity Assay:

1. TNB Standard Curve:

Add 0, 2, 4, 6, 8, 10 µl of the TNB Standard into 96-well plate in duplicate to generate 0, 10, 20, 30, 40, 50 nmol/well standard. Bring the final volume to 100 µl with Assay Buffer.

2. Sample and Positive Control Preparations:

Take 20 mg Tissue or 2 x 10^6 Cells and homogenize in 100-200 µl cold Assay Buffer on ice (It is recommended to add Protease Inhibitor Cocktail to the buffer); Centrifuge at 10,000 x g for 15 min at 4°C, Collect the supernatant for assay and store on ice.

3. Serum can be tested directly. Determine the protein concentration of the supernatant using the Bradford Reagent. Keep samples at -80°C for storage.

4. Assay Procedure: Add 2 - 50 µl sample or 10 µl TrxR positive control into each well, adjust volume to 50 µl with assay buffer. 2 sets of samples should be tested as with or without TrxR Inhibitor. Add 10 µl of TrxR Inhibitor to one set of the sample for testing background enzyme activity, and add 10 µl of Assay Buffer to the other set of sample for teting total DTNB reduction, mix well.

5. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 40 µl Reaction Mix:

Assay Buffer	30 µl
DTNB Solution	8 µl
NADPH	2 µl

6. Add 40 µl of the Reaction Mix to each test sample, mix well. Measure OD 412 nm at T1 to get A1t and A1I, measure OD 412 nm again at T2 after incubating the reaction at 25°C for 20 min (The incubate time can vary depend on the sample concentration) to get A2t and A2I, protect from light. The OD of TNB2- generated by TrxR is ΔA412 nm = (A2t - A1t) - (A2I - A1I).

Note: It is essential to read A1t, A1I, A2t and A2I' in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose A1t, A1I, A2t and A2I in the reaction linear range.

7. Calculation: Plot the TNB standard Curve. Apply the ΔA412nm to the TNB standard curve to get B nmol of TNB (TNB amount generated between T1 and T2 in the reaction wells).

TrxR Activity = B/[(T2-T1) × V] × Sample Dilution Factor = nmol/min/ml = mU/mL

Where: B is the TNB amount from TNB standard Curve (in nmol).

T1 is the time of the first reading (A1t and A1I) (in min).

T2 is the time of the second reading (A2t and A2I) (in min).

V is the pretreated sample volume added into the reaction well (in ml).

TrxR Unit Definition: One unit of TrxR is the amount of enzyme that generates1.0 µmol of TNB per minute at 25°C. The oxidation of 1 mole of NADPH to NADP will generate 2 mole TNB finally, therefore, 1 TNB unit equals 0.5 NADP unit.

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates;

		Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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Our promise

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