CREATIVE BIOMART®
Assay Kit

Thioredoxin Reductase Activity Assay Kit

Product Information

Cat.No. Kit-0833

Product Overview

Thioredoxin Reductase Assay Kit provides a convenient colorimetric assay for detecting TrxR activity in various samples. In the assay TrxR catalyzes the reduction of 5, 5'-dithiobis (2-nitrobenzoic) acid (DTNB) with NADPH to 5-thio-2-nitrobenzoic acid (TNB2-), which generates a strong yellow color (λmax = 412 nm). Since in crude biological samples other enzymes, such as glutathione reductase and glutathione peroxidase can also reduce DTNB, therefore, TrxR specific inhibitor is utilized to determine TrxR specific activity. Two assays are performed. Tthe first measurement is of the total DTNB reduction by the sample, and the second one is the DTNB reduction by the sample in the presence of the TrxR specific inhibitor. The difference between the two results is the DTNB reduction by TrxR.

Storage

Store the kit at -20°C, protect from light. Warm TrxR Assay Buffer to room temperature (RT) before use. Briefly centrifuge vials prior to opening.

Size

100 assays

Kit Components

TrxR Assay Buffer 25 ml
TNB Standard (lyophilized) 1 vial
DTNB (lyophilized) 1 vial
NADPH (lyophilized) 1 vial
TrxR Positive Control 1 vial
TrxR Inhibitor (lyophilized) 1 vial

Preparation

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

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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 months at -20°C.
NADPH: Dissolve one vial with 0.22 ml dH2O; sufficient for 100 assays. The solution is stable for 1 week at 4°C or 2 months 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 months 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 months at -20°C. Ensure that the Assay Buffer is at RT before use. Keep samples, NADPH, TrxR inhibitor, TrxR Positive Control on ice during the assay.

Assay Protocol

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 106 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 samples 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 testing 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:
30 µl Assay Buffer
8 µl DTNB Solution
2 µl NADPH
6. Add 40 µl of the Reaction Mix to each test sample, mix well. Measure OD 412 nm at T1 to get A1t and A1l, measure OD 412 nm again at T2 after incubating the reaction at 25°C for 20 min (The incubation time can vary depending on the sample concentration) to get A2t and A2l, protect

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from light. The OD of TNB2- generated by TrxR is ΔA412 nm = (A2t – A1t) - (A2l - A1l).
Note: It is essential to read A1t, A1l, A2t and A2l' in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose A1t, A1l, A2t and A2l 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) x V] x 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 A1l) (in min).
T2 is the time of the second reading (A2t and A2l) (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.

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