

Thioredoxin Reductase Assay Kit

(Catalog #K763-100; 100 reactions; Store kit at -20°C)

I. Introduction:

Thioredoxin reductase (TrxR) (EC 1.8.1.9) is a ubiquitous enzyme which is involved in many cellular processes such as cell growth, p53 activity, and protection against oxidation stress, etc. The mammalian TrxR reduces thioredoxins as well as non-disulfide substrates such as selenite, lipoic acids, lipid hydroperoxides, and hydrogen peroxide. BioVision's 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 generate a strong yellow color ($\lambda_{max} = 405 \text{ nm}$). 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: the 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.

II. Kit Contents:

Components	K763-100	Cap Code	Part No.
TrxR Assay Buffer	25 ml	WM	K763-100-1
TNB Standard	1 vial	Yellow	K763-100-2
DTNB	1 vial	Red	K763-100-3
NADPH	1 vial	Blue	K763-100-4
TrxR Positive Control (~20mU)	1 vial	Green	K763-100-5
TrxR Inhibitor	1 vial	White	K763-100-6

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 1 week at 4°C or 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 dH₂O; sufficient for 100 assays. The solution is stable for 1 week at 4°C or 2 month at -20°C.

TrxR Positive Control: Dilute 10 μl TrxR 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. Glutathione 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 Tissues or 2x10⁶ Cells and homogenize in 100-200 μl cold Assay Buffer on ice (It is recommended to add Protease Inhibitor Cocktail (Cat.# K271-500) 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 (BioVision Cat # K810-100). 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 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 O.D.405 nm at T₁ to get A_{1t} and A_{1i}, measure O.D.405 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 A_{2t} and A_{2i}, protect from light. The O.D. of TNB²⁻ generated by TrxR is $\Delta A_{405 \text{ nm}} = (A_{2t} - A_{1t}) - (A_{2i} - A_{1i})$.

Note: It is essential to read A_{1t}, A_{1i}, A_{2t} and A_{2i} in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose A_{1t}, A_{1i}, A_{2t} and A_{2i} in the reaction linear range.

7. **Calculation:** Plot the TNB standard Curve. Apply the $\Delta A_{405 \text{ nm}}$ to the TNB standard curve to get B nmol of TNB (TNB amount generated between T1 and T2 in the reaction wells).

$$\text{TrxR Activity} = \frac{B}{(T_2 - T_1) \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/mL}$$

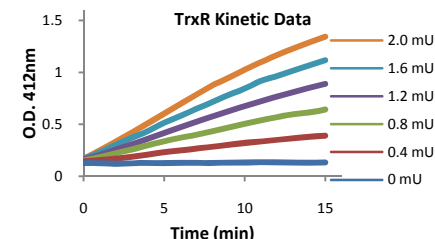
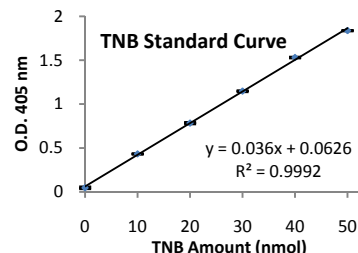
Where: B is the TNB amount from TNB standard Curve (in nmol).

T₁ is the time of the first reading (A_{1t} and A_{1i}) (in min).

T₂ is the time of the second reading (A_{2t} and A_{2i}) (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 generates 1.0 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.



VI. Related Products:

Colorimetric Glutathione Detection Kit
Glutathione Kit (GSH, GSSG and Total)
GST Colorimetric Assay Kit
Acid/Alkaline Phosphatase Assay Kit
Phosphate Assay Kit
NADP/NADPH Quantitation Kit
Pyruvate Assay Kit
Ammonia Assay Kit
Glycogen Assay Kit

GST Fluorometric Assay Kit
Fatty Acid Assay Kit
Triglyceride Assay Kit
ADP/ATP Ratio Assay Kit
NAD/NADH Quantification Kit
Glucose Assay Kit
Lactate Assay Kit/ II
Glutamate Assay Kit
Ethanol Assay Kit