ab83463

Thioredoxin Reductase (TrxR) Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Thioredoxin Reductase activity in various samples

This product is for research use only and is not intended for diagnostic use.

Version: 6 Last Updated: 29 January 2019

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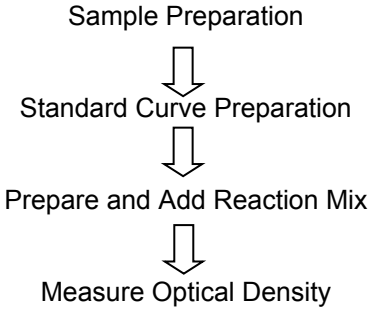
1. Overview

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.

Abcam's Thioredoxin Reductase (TrxR) 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 (TNB^{2-}), which generates a strong yellow color (λ_{max} = 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: 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.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
TrxR Assay Buffer	25 mL
TNB Standard (2.5 μmol) (Lyophilized)	1 vial
DTNB (Lyophilized)	1 vial
NADPH (Lyophilized)	1 vial
TrxR Positive Control (~20 mU; Lyophilized)	1 vial
TrxR Inhibitor (Lyophilized)	1 vial

* Store kit at -20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Keep samples, NADPH, TrxR inhibitor, TrxR Positive Control on ice during the assay. Read the entire protocol before performing the assay.

TNB STANDARD: Dissolve 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 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 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.

B. Additional Materials Required

- Microcentrifuge
- Bradford Reagent (**ab102535**)
- Protease Inhibitor Cocktail (**ab65621**)
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96-well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

Take 20 mg tissue or 2 x 10^6 cells and homogenize in 100-200 µL cold Assay Buffer on ice. Centrifuge at 10,000 x g for 15 min at +4°C; Collect the supernatant for assay and store on ice. Serum can be tested directly.

Determine the protein concentration of the supernatant using the Bradford Reagent (**ab102535**). Keep samples at -80°C for storage.

Note:

It is recommended to add Protease Inhibitor Cocktail (**ab65621**) to the buffer.

Add 2-50 µL sample or 10 µL TrxR positive control into each well, adjusting volume to 50 µL with assay buffer.

2 sets of samples should be tested 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.

2. 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.

3. 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

Add 40 µL of the Reaction Mix to each test sample, mix well.

4. Measure OD_{412nm} at T_1 to get A_{1t} and A_{1I}, measure OD_{412nm} again at T_2 after incubating the reaction at 25°C for 20 min, to get A_{2t} and A_{2I}, protect from light. The incubation times can vary depending on the sample concentration.

The OD of TNB^{2-} generated by TrxR is:

$$\mathbf{\Delta A_{412nm} = (A_{2AB} - A_{2INH}) - (A_{1AB} - A_{1INH})}$$

where AB is assay buffer, INH is inhibitor

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.

Data Analysis

Plot the TNB standard curve. Apply the ΔA_{412nm} to the TNB standard curve to get ΔB nmol of TNB.

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

Where:

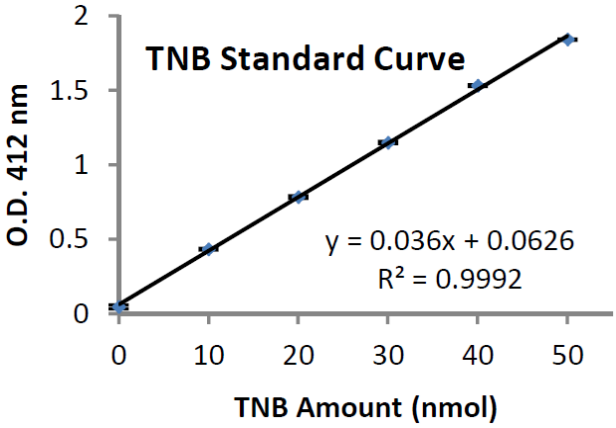
ΔB is the TNB amount from TNB standard curve (in nmol).

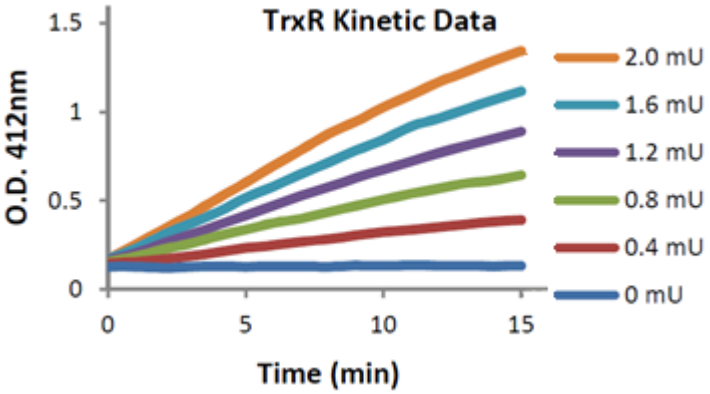
T_1 is the time of the first reading (A_{1t}, and A_{1l}) (in min).

T_2 is the time of the second reading (A_{2t} and A_{2l}) (in min).

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

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 mol of NADPH to NADP will generate 2 mol TNB finally, therefore, 1 TNB unit equals 0.5 NADP unit.


TNB Standard Curve
O.D. 412 nm
TNB Amount (nmol)
y = 0.036x + 0.0626
R² = 0.9992
0
0.5
1
1.5
2
0
10
20
30
40
50


TrxR Kinetic Data
O.D. 412nm
Time (min)
2.0 mU
1.6 mU
1.2 mU
0.8 mU
0.4 mU
0 mU
0
0.5
1
1.5
0
5
10
15

5. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp