

**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.

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# 1. Overview

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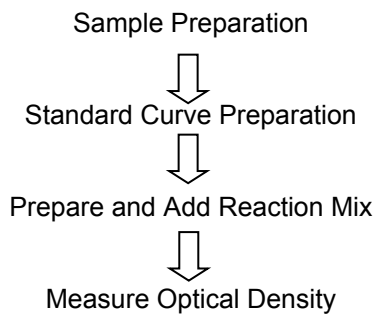
Thioredoxin reductase (TrxR) (EC 1.8.1.9) is a ubiquitous enzyme which is involved in many cellular processes such as cell growth, p53 activity, and protection against oxidation stress, etc. The mammalian TrxR reduces thioredoxins as well as non-disulfide substrates such as selenite, lipoic acids, lipid 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 I to 5-thio-2-nitrobenzoic acid (TNB<sup>2-</sup>), which generates a strong yellow color ( $\lambda_{\text{max}} = 412 \text{ nm}$ ). 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.

## 2. Protocol Summary

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### 3. Components and Storage

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#### A. Kit Components

| <b>Item</b>                            | <b>Quantity</b> |
|--|-----------------|
| Thioredoxin Reductase Assay Buffer     | 25 mL           |
| TNB Standard                           | 1 vial          |
| DTNB                                   | 1 vial          |
| NADPH I                                | 1 vial          |
| Thioredoxin Reductase Positive Control | 10 $\mu$ L      |
| Thioredoxin Reductase Inhibitor        | 1 vial          |

PLEASE NOTE: NADPH I was previously labelled as NADPH (Lyophilized), and Thioredoxin Reductase Assay Buffer as TrxR Assay Buffer, and Thioredoxin Reductase Inhibitor as TrxR Inhibitor (Lyophilized), and Thioredoxin Reductase Positive Control as TrxR Positive Control. The composition has not changed.

\* Store kit at  $-20^{\circ}\text{C}$ , protect from light. Warm Thioredoxin Reductase Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Keep samples, NADPH I, Thioredoxin Reductase Inhibitor, Thioredoxin Reductase Positive Control on ice

during the assay. Read the entire protocol before performing the assay.

**TNB STANDARD:** Dissolve TNB Standard into 0.5 mL Thioredoxin Reductase Assay Buffer to generate 5 mM TNB Standard. Store at -20°C.

**DTNB SOLUTION:** Dissolve DTNB into 0.9 mL Thioredoxin Reductase Assay Buffer, sufficient for 100 assays. Briefly warm the solution in a 37°C water bath or heat block, and sonicate or vortex to bring fully into solution. Store at -20°C.

**NADPH I:** Dissolve one vial with 0.22 mL dH<sub>2</sub>O; sufficient for 100 assays. Store at -20°C.

**Thioredoxin Reductase Positive Control:** Resuspend with 90 µL Thioredoxin Reductase Assay Buffer; it is stable for 1 day at +4°C otherwise store at -20°C.

**Thioredoxin Reductase Inhibitor:** Dissolve Thioredoxin Reductase Inhibitor into 1.2 mL Thioredoxin Reductase Assay Buffer, sufficient for 100 assays. The Thioredoxin Reductase 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

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### 1. Sample Preparation:

Take 20 mg tissue or  $2 \times 10^6$  cells and homogenize in 100-200  $\mu\text{L}$  cold Thioredoxin Reductase Assay Buffer on ice. Centrifuge at  $10,000 \times g$  for 15 min at  $+4^\circ\text{C}$ ; Collect the supernatant for assay and store on ice. Serum can be tested directly.

Determine the protein concentration of the cell or tissue supernatant using the Bradford Reagent (**ab102535**). Keep samples at  $-80^\circ\text{C}$  for storage.

### Note:

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

Add 2-50  $\mu\text{L}$  sample or 10  $\mu\text{L}$  Thioredoxin Reductase Positive Control into each well, adjusting volume to 50  $\mu\text{L}$  with Thioredoxin Reductase Assay Buffer.

2 sets of samples should be tested with or without Thioredoxin Reductase Inhibitor. Add 10  $\mu\text{L}$  of Thioredoxin Reductase Inhibitor to one set of the sample for testing background enzyme activity, and add 10  $\mu\text{L}$  of Thioredoxin Reductase 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  $\mu\text{L}$  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  $\mu\text{L}$  with Thioredoxin Reductase Assay Buffer.

## 3. Reaction Mix:

Mix enough reagents for the number of assays to be performed. For each well, prepare a total 40  $\mu\text{L}$  Reaction Mix:

|                                    |                  |
|------------------------------------|------------------|
| Thioredoxin Reductase Assay Buffer | 30 $\mu\text{L}$ |
| DTNB Solution                      | 8 $\mu\text{L}$  |
| NADPH I                            | 2 $\mu\text{L}$  |

Add 40  $\mu\text{L}$  of the Reaction Mix to each test sample and positive control, mix well.

4. Measure  $\text{OD}_{412\text{nm}}$  at  $T_1$  to get  $A_{1t}$  and  $A_{1i}$ , measure  $\text{OD}_{412\text{nm}}$  again at  $T_2$  after incubating the reaction at  $25^\circ\text{C}$  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  $\text{TNB}^{2-}$  generated by TrxR is:

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

where AB is Thioredoxin Reductase Assay Buffer, INH is inhibitor

### 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.

## Data Analysis

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Plot the TNB standard curve. Apply the  $\Delta A_{412\text{nm}}$  to the TNB standard curve to get  $\Delta B$  nmol of TNB.

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

Where:

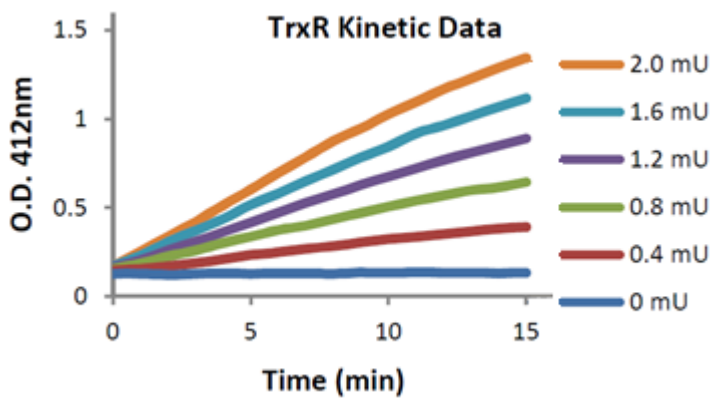
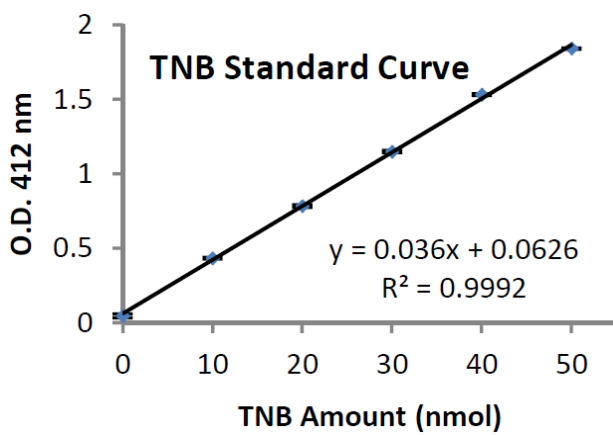
**$\Delta B$**  is the TNB amount from TNB standard curve (in nmol).

**$T_1$**  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  $\mu\text{mol}$  of TNB per minute at 25°C. The oxidation of 1 mol of NADPH I to NADP will generate 2 mol TNB finally, therefore, 1 TNB unit equals 0.5 NADP unit.



## 5. Troubleshooting

| <b>Problem</b>     | <b>Reason</b>   | <b>Solution</b>   |
|--------------------|---|---|
| Assay not working  | Thioredoxin Reductase Assay Buffer at wrong temperature | Thioredoxin Reductase 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);<br>Luminescence: White plates;<br>Colorimetry: Clear plates.<br>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 Thioredoxin Reductase 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) |

| <b>Problem</b>               | <b>Reason</b>  | <b>Solution</b>  |
|------------------------------|--|--|
| 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   |

## **Technical Support**

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