Thioredoxin Reductase Colorimetric Assay Kit

Item No. 10007892

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

TABLE OF CONTENTS

GENERAL INFORMATION
3 Materials Supplied
4 Safety Data
4 Precautions
4 If You Have Problems
5 Storage and Stability
5 Materials Needed but Not Supplied

INTRODUCTION
6 Background
7 About This Assay

PRE-ASSAY PREPARATION
8 Reagent Preparation
10 Sample Preparation

ASSAY PROTOCOL
12 Plate Set Up
14 Performing the Assay

ANALYSIS
15 Calculations
17 Performance Characteristics

RESOURCES
18 Interferences
19 Troubleshooting
20 References
21 Plate Template
22 Notes
23 Warranty and Limitation of Remedy

GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity	Storage Temperature
10009092	TrxR Assay Buffer (10X)	1 vial/5 ml	-20°C
400696	TrxR Positive Control	1 vial/100 µl	-20°C
10009094	TrxR Inhibitor	2 vials	-20°C
760912	DTNB Assay Reagent	2 vials	-20°C
10009095	TrxR NADPH	2 vials	-20°C
400014	96-Well Plate (colorimetric assay)	1 plate	RT
400012	96-Well Cover Sheet	1 cover	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Thioredoxin Reductase Colorimetric Assay Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the Materials Supplied section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 405 nm
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of pure water; glass distilled water or water is acceptable. *NOTE: Ultrapure Water is available for purchase from Cayman (Item No. 400000).*
4. Materials needed for **Sample Preparation** (see page 10)

INTRODUCTION

Background

Thioredoxin reductases (TrxRs) are selenium-containing pyridine nucleotide-disulfide oxidoreductases and part of the antioxidant thioredoxin system that is involved in the maintenance of cellular redox homeostasis.[1-3] There are three isoforms of TrxR: TrxR1, which is localized to the cytosol, and TrxR2 and TrxR3, which are localized to the mitochondria.[4] All TrxR isoforms catalyze the NADPH-dependent reduction of oxidized Trx and other oxidized protein disulfide substrates, as well as selenite lipid hydroperoxides, vitamin K, and hydrogen peroxide.[1,2,4-7] TrxRs regulate several redox-sensitive biological processes, including apoptosis and cell growth, proliferation, and survival, and have been implicated in the pathology of cancer, neurodegenerative disorders, chronic inflammatory diseases, autoimmune diseases, and parasitoses.[4,8-10]

About This Assay

Cayman's Thioredoxin Reductase Colorimetric Assay Kit provides a convenient method for detecting TrxR activity in tissue homogenates and cell lysates. It is based on the reduction of DTNB (5,5'-dithio-*bis*(2-dinitrobenzoic acid); Ellman's reagent) with NADPH to 5-thio-2-nitrobenzoic acid (TNB) which produces a yellow product that is measured at 405 nm.[11,12] The kit includes all reagents needed to assay mammalian TrxR activity. Measurement of TrxR activity by DTNB reduction in the absence and in the presence of aurothiomalate, a specific TrxR inhibitor included in the kit, allows for correction of non-thioredoxin reductase-independent DTNB reduction (*i.e.*, presence of glutathione).[13] The difference between the two results is the DTNB reduction due to TrxR activity.

$$TrxR + DTNB + NADPH + H^+ \longrightarrow 2TNB + NADP^+$$

PRE-ASSAY PREPARATION

Reagent Preparation

1. **TrxR Assay Buffer (10X) - (Item No. 10009092)**

   This vial contains 5 ml of TrxR Assay Buffer (10X). Thaw at room temperature and dilute 3 ml with 27 ml of pure water. The TrxR Assay Buffer (1X) will be stable for six months when stored at -20°C.

2. **TrxR Positive Control - (Item No. 400696)**

   The vial contains 100 µl of rat recombinant thioredoxin reductase 1. Immediately prior to use in the assay, thaw on ice. Once thawed, it is ready to use as supplied. If not using all at once, prepare aliquots and store at -20°C. Limit freeze-thaw cycles to two.

3. **TrxR Inhibitor - (Item No. 10009094)**

   Each vial contains a lyophilized powder of sodium aurothiomalate (ATM), a thioredoxin reductase inhibitor. Reconstitute one vial with 1 ml of TrxR Assay Buffer (1X) and place on ice, where it will be stable for four hours. One vial provides a sufficient amount to assay 50 wells. ATM is used to determine the thioredoxin reductase-independent reduction of DTNB in samples. The concentration of inhibitor used in the assay, 20 µM, will effectively remove all thioredoxin reductase activity.[14]

4. **DTNB Assay Reagent - (Item No. 760912)**

   Each vial contains a lyophilized powder of DTNB. Reconstitute one vial with 2 ml of TrxR Assay Buffer (1X). This is a sufficient volume to assay 100 wells. If additional wells will be utilized, reconstitute the second vial. The reconstituted DTNB Assay Reagent will be stable for four hours at room temperature. Keep protected from light.

5. **TrxR NADPH - (Item No. 10009095)**

   Each vial contains a lyophilized powder of NADPH. Reconstitute one vial with 2 ml of TrxR Assay Buffer (1X) and place on ice, where it will be stable for six hours. One vial provides a sufficient amount to assay 100 wells. If additional wells will be utilized, reconstitute the second vial.

Sample Preparation

Samples should be processed and assayed immediately after collection; samples that cannot be assayed immediately should be frozen and stored at -80°C. To fall within the range of the assay, it may be necessary to dilute samples several times with TrxR Assay Buffer (1X) prior to the assay.

Tissue Homogenate

The amount of TrxR activity in animal tissues varies from organ to organ. Values range from 0.05-0.6 units per mg of protein for crude extracts.

1. Prior to dissection, either perfuse tissue or rinse tissue with a phosphate buffered saline (PBS) solution, pH 7.4, to remove any red blood cells and clots.
2. Homogenize the tissue in 2-5 ml of cold buffer (*i.e.*, 50 mM potassium phosphate, pH 7.4, containing 1 mM EDTA) per gram of tissue.
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Transfer the supernatant to a new tube and store on ice or at -80°C for long-term storage.

Cell Lysate

Typically, cell culture extracts have a range of 0.4-4 units per 10^8 cells (0.04-0.25 units per mg of protein).

1. Collect 2 x 10^6 cells by centrifugation. For adherent cells, do not harvest using proteolytic enzymes, rather use a cell scraper.
2. Add 200 µl cold buffer (*i.e.*, 50 mM potassium phosphate, pH 7.4, containing 1 mM EDTA) and ~100 mg glass beads (500-750 µm) to the cell pellet.
3. Vortex for 30 seconds and chill on ice for 30 seconds. Repeat five times.
4. Centrifuge for 15 minutes at 15,000 x g at 4°C.
5. Transfer the supernatants to a new tube and store on ice or at -80°C for long-term storage.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, it is necessary to designate two wells each for the positive control reactions. It is strongly recommended that sample background wells are run to determine TrxR-independent DTNB reduction.

A typical layout of samples to be measured in duplicate is given below (see Figure 1). It is suggested that the contents of each well are recorded on the template sheet provided on page 21.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	PC	S7	S7	S15	S15	S23	S23	B8	B8	B16	B16
B	NC	NC	S8	S8	S16	S16	B1	B1	B9	B9	B17	B17
C	S1	S1	S9	S9	S17	S17	B2	B2	B10	B10	B18	B18
D	S2	S2	S10	S10	S18	S18	B3	B3	B11	B11	B19	B19
E	S3	S3	S11	S11	S19	S19	B4	B4	B12	B12	B20	B20
F	S4	S4	S12	S12	S20	S20	B5	B5	B13	B13	B21	B21
G	S5	S5	S13	S13	S21	S21	B6	B6	B14	B14	B22	B22
H	S6	S6	S14	S14	S22	S22	B7	S7	B15	B15	B23	B23

PC = Positive Control Wells
NC = Negative Control Wells
S1-S23 = Sample Wells
B1-B23 = Sample Background Wells

Figure 1. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps to maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 200 µl in all the wells.
- All reagents should be prepared as described above. The TrxR Positive Control, TrxR Inhibitor, and TrxR NADPH should be kept on ice and all other reagents should be kept at room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples be assayed at least in duplicate (triplicate is preferred), but it is at the user's discretion to do so.
- The assay is performed at room temperature (22°C to 25°C).
- Twenty-three samples can be assayed in duplicate.
- Monitor the absorbance at 405 nm.

Performing the Assay

1. Add the reagents to the designated wells following the table below.

Reagent	Positive Control Wells	Negative Control Wells	Sample Wells	Sample Background Wells
TrxR Assay Buffer (1X)	140 µl	120 µl	140 µl	120 µl
Reconstituted TrxR Inhibitor	--	20 µl	--	20 µl
TrxR Positive Control	20 µl	20 µl	--	--
Sample	--	--	20 µl	20 µl

Table 1. Pipetting summary

2. Initiate the reactions by adding 20 µl of NADPH and 20 µl of DTNB to all the wells being used. Carefully shake the plate for 30 seconds to mix.
3. Read the absorbance once every minute at 405 nm using a plate reader to obtain at least ten time points.

ANALYSIS

Calculations

Determination of the Reaction Rate

1. Determine the change in absorbance per minute (abs/min) for each well by either:
   a. Plotting the average absorbance values as a function of time to obtain the slope (rate) of the linear portion of the curve (a graph is shown using rat recombinant TrxR, see Figure 2, page 16) - *or*-
   b. Selecting two points on the linear portion of the curve and determine the change in absorbance during that time using the following equation:

$$\text{abs/min} = \frac{A_{405}\ (\text{Time 2}) - A_{405}\ (\text{Time 1})}{\text{Time 2 (min)} - \text{Time 1 (min)}}$$

2. Calculate the average rate of the sample and sample background wells.
3. Determine the inhibitor-corrected rate (ICR) of each sample using the equation below.

$$ICR_{sample}\ (\text{abs/min}) = \text{Sample Rate} - \text{Sample Background Rate}$$

4. Calculate the TrxR activity in U/ml using the equation below. One unit is defined as the NADPH-dependent production of 2 µmol of 2-nitro-5-thiobenzoate per minute under the conditions specified in this assay.

$$\text{TrxR Activity}\left(\frac{U}{ml}\right) = ICR\left(\frac{abs}{min}\right) \div 7.92\ mM^{-1} \times \frac{0.2\ ml}{0.02\ ml} \times \text{Dilution Factor}$$

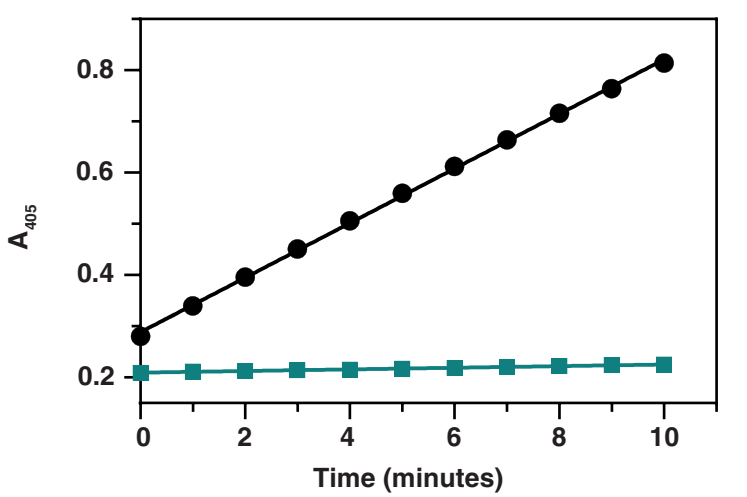


Figure 2. Activity of the TrxR Positive Control

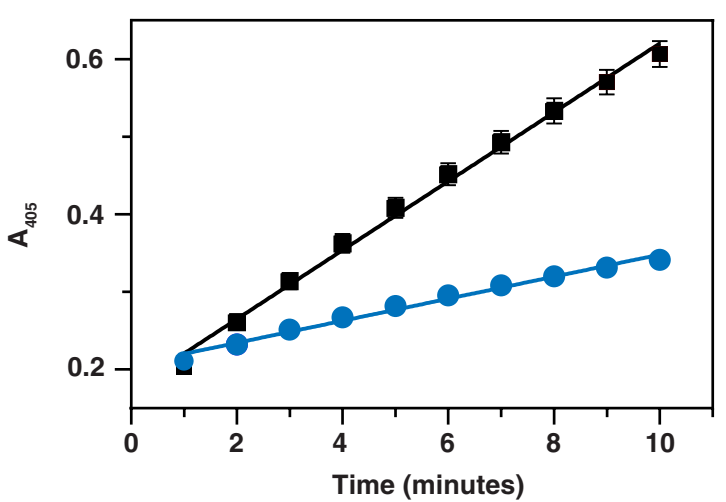


Figure 3. Thioredoxin reductase activity in cell and tissue lysates

Performance Characteristics

Sensitivity:

Under the standardized conditions of the assay described in this booklet, the detection range of the assay is from 0.008-0.09 µmol/min/ml of TrxR activity, which is equivalent to an absorbance increase of 0.006-0.07 per minute.

Precision:

When a series of ten thioredoxin reductase measurements were performed on the same day, the intra-assay coefficient of variation was 3.6%. When a series of eleven thioredoxin reductase measurements were performed on eight different days under the same experimental conditions, the inter-assay coefficient of variation was 12%.

RESOURCES

Interferences

The following reagents were tested for interference in the assay.

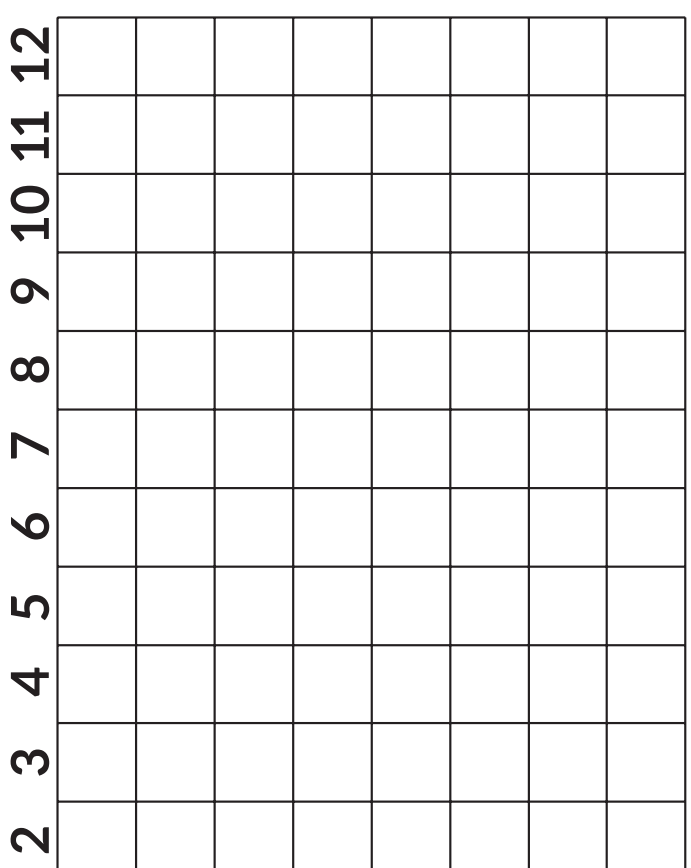
	Reagent	Will Interfere (Yes or No)
Buffers:	Tris (pH 8)	No
	HEPES (pH 7)	No
	Phosphate (pH 7)	No
	Borate (pH 9)	Yes
Detergents:	Triton X-100 (≤1%)	No
	Polysorbate 20 (≤1%)	No
	CHAPS (≤1%)	No
Protease Inhibitors/ Chelators:	Antipain (≤0.1 mg/ml)	No
	PMSF (≤200 µM)	No
	Leupeptin (≤10 µg/ml)	No
	Trypsin (≤10 µg/ml)	No
	Chymostatin (≤10 µg/ml)	No
	EDTA (≤10 mM)	No
	EGTA (≤1 mM)	No
Solvents:	Ethanol (10 µl)	No
	Methanol (10 µl)	Yes
	Dimethylsulfoxide (10 µl)	No
Others:	Glutathione	Yes
	BSA (≤1%)	No
	Glycerol (≤10%)	No

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of replicates	A. Poor pipetting/technique B. Bubble(s) in the well	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No activity was detected in the sample	A. There is no TrxR in the sample B. The TrxR activity is too low C. NADPH and/or DTNB was not added to the sample well	A,C. Perform the assay again to verify B. Concentrate the sample using a spin concentrator with a MWCO of 3 kDa and assay again OR if sample was dilute, use a lower dilution and assay again
No activity was observed in the positive control	A. The TrxR positive control was not added to the well B. The NADPH and/ or DTNB solutions were not added to the wells	A, B. Repeat the experiment and make sure to add all reagents to the appropriate wells
The sample starting absorbance is >1.0	There may be interference due to thiol-containing compounds	Dilute the sample and re-assay

References

1. Miranda-Vizuete, A., Damdimopoulos, A.E., and Spyrou, G. *Antioxid. Redox Signal.* **2(4)**, 801-810 (2000).
2. Cebula, M., Schmidt, E.E., and Arnér, E.S.J. TrxR1 as a potent regulator of the Nrf2-Keap1 response system. *Antioxid. Redox Signal.* **23(10)**, 823-853 (2015).
3. Watanabe, R., Nakamura, H., Masutani, H., *et al. Pharmacol. Ther.* **127(3)**, 261-270 (2010).
4. Bjørklund, G., Zou, L., Wang, J., *et al.* Thioredoxin reductase as a pharmacological target. *Pharm. Res.* **174**, 105854 (2021).
5. Li, W., Bandyopadhyay, J., Hwaang, H.S., *et al. Mol. Cells* **34(2)**, 209-218 (2012).
6. Mustacich, D. and Powis, G. *Biochem. J.* **346(Pt. 1)**, 1-8 (2000).
7. Becker, K., Gromer, S., Schirmer, R. H., *et al. Eur. J. Biochem.* **267**, 6118-6125 (2000).
8. Chen, G., Chen, Q., Zeng, F., *et al. Oncotarget* **8(70)**, 115270-115279 (2017).
9. Holzerova , E., Danhauser, K., Haack, T.B., *et al. Brain* **139(Pt. 2)**, 346-354 (2016).
10. Zhang, R., Al-Lamki, R., Bai, L., *et al. Circ. Res.* **94(11)**, 1483-1491 (2004).
11. Smith, A.D., Morris, V.C., and Levander, O.A. *Int. J. Vitam. Nutr. Res.* **71(1)**, 87-92 (2001).
12. Hill, K.E., McCollum, G.W., and Burk, R.F. *Anal. Biochem.* **253**, 123-125 (1997).
13. Smith, A.D., Guidry, C.A., Morris, V.C., *et al. J. Nutr.* **129**, 194-198 (1999).
14. Gromer, S., Arscott, L. D., Williams, C. H., Jr., *et al. J. Biol. Chem.* **273(32)**, 20096-20101 (1998).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

This document is copyrighted. All rights are reserved. This document may not, in whole or part, be copied, photocopied, reproduced, translated, or reduced to any electronic medium or machine-readable form without prior consent, in writing, from Cayman Chemical Company.

©02/13/2025, Cayman Chemical Company, Ann Arbor, MI, All rights reserved. Printed in U.S.A.