abcam

Product datasheet

Thioredoxin Reductase Assay Kit (Colorimetric) ab83463

28 References 5 Images

Overview

Product name	Thioredoxin Reductase Assay Kit (Colorimetric)
Detection method	Colorimetric
Sample type	Urine, Cell culture extracts, Other biological fluids, Tissue Extracts
Assay type	Enzyme activity
Assay time	0h 40m
Product overview	Thioredoxin Reductase Assay Kit (ab83463) is a specific assay for detecting Thioredoxin Reductase (TrxR) activity. In the thioredoxin reductase assay protocol, TrxR catalyzes the reduction of DTNB to TNB^{2-} in the presence of NADPH, which generates a strong yellow color (ODmax = 412 nm). Other enzymes present in crude biological samples such as glutathione reductase and glutathione peroxidase can also reduce DTNB. In order to measure TrxR-only activity, a TrxR specific inhibitor is used in a separate reaction to determine TrxR specific activity. The difference between total DTNB reduction in the sample and DTNM reduction in the sample in presence of TrxR inhibitor is the value of specific TrxR activity in the sample. **We have tested the GR positive control from ab83461 in the conditions of ab83463, and we do not observe activity of the GR positive control.** Thioredoxin reductase assay protocol summary: - add samples and standards to wells - add reaction mix - analyze with a microplate reader over 20 min
Notes	This product is manufactured by BioVision, an Abcam company and was previously called K763 Thioredoxin Reductase Activity Colorimetric Assay Kit. K763-100 is the same size as the 100 test size of ab83463. Thioredoxin reductase (TrxR, EC 1.8.1.9) is a ubiquitous mammalian enzyme that catalyzes the NADPH-dependent reduction of the redox protein thioredoxin, as well as of other endogenous and exogenous compounds such as selenite, lipid hydroperoxides and hydrogen peroxide.

Platform Microplate reader

Properties

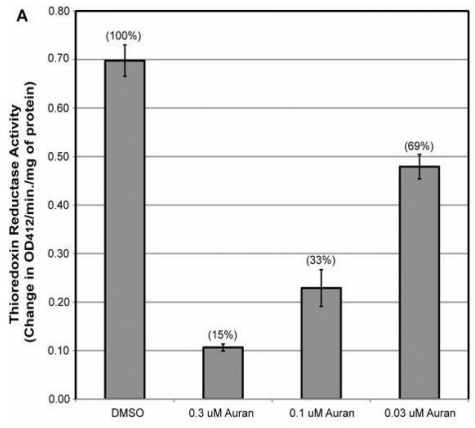
Storage instructions Store at -20°C. Please refer to protocols.

Components	100 tests
DTNB	1 vial
NADPH I	1 vial
Thioredoxin Reductase Assay Buffer	1 x 25ml
Thioredoxin Reductase Inhibitor	1 vial
Thioredoxin Reductase Positive Control	1 vial
TNB Standard	1 vial

Relevance 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.

Cellular localization TXNRD1: Cytoplasmic. TXNRD2: Cytoplasmic. Nuclear. Microsome. Endoplasmic reticulum. TXNRD3: Mitochondrial.

Images



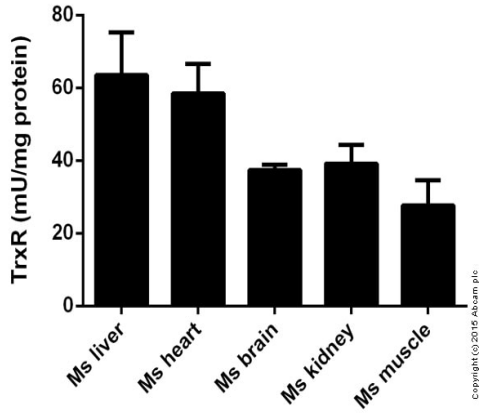
Bulman CA et al. PLoS One. 9:e0003534 (2015)

Functional Studies - beta Thioredoxin reductase Assay Kit (ab83463)

Activity of endogenous *Brugia* thioredoxin reductase from soluble worm lysates following incubation with 1% DMSO or 0.3 µM, 0.1 µM, or 0.03 µM of auranofin *in vitro*. Percentages indicate the percent activity of TrxR compared to DMSO controls. Thioredoxin reductase activity was significantly reduced ($p < 0.05$) to 15%, 33% and 69% of endogenous activity, respectively, compared to the activity in DMSO-treated worms.

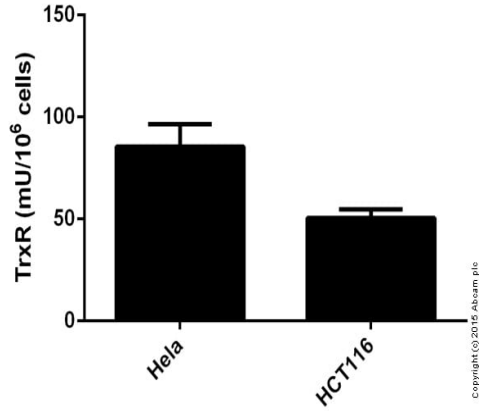
Thioredoxin reductase activity of worm lysates was assayed using female *B. malayi* treated *in vitro* with either 0.3 µM, 0.1 µM, or 0.03 µM auranofin or 1% DMSO. After 5 hours of treatment, worm motility was measured using the Worminator, and then worms (24 in each group) were pooled, washed three times in PBS, and lysed by douncing in a glass homogenizer in assay buffer (ab83463) with 1 mM PMSF. The crude lysates were centrifuged at 10,000 rcf for 15 minutes at 4°C to pellet insoluble material. The total protein concentrations of soluble lysates were measured using the Bradford assay. The soluble lysates were incubated for 20 minutes in assay

buffer or assay buffer with a proprietary thioredoxin reductase specific inhibitor before adding a specific substrate, DTNB (5, 5′-dithiobis (2-nitrobenzoic) acid), and measuring activity at 20 second intervals for 40 minutes using the SpectraMax Plus Microplate Reader (Molecular Devices, Sunnyvale, CA) at λ = 412 nm. Lysates were tested in duplicate. TrxR activity was calculated based on the linear amount of TNB produced per minute per mg of total protein and adjusted for background activity from enzymes other than TrxR in the lysates.



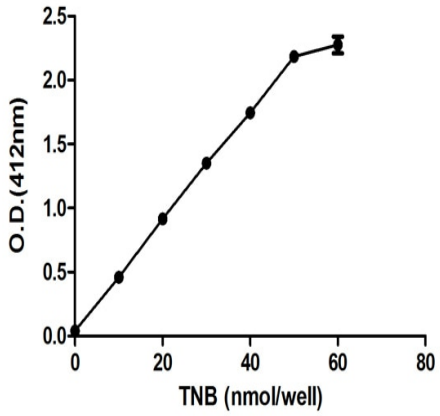
Functional Studies - beta Thioredoxin reductase Assay Kit (ab83463)

Thioredoxin reductase measured in mouse tissue lysates showing activity (mU) per mg protein of sample tested



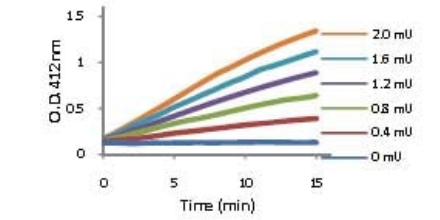
Functional Studies - beta Thioredoxin reductase Assay Kit (ab83463)

Thioredoxin reductase measured in cell lysates showing activity (mU) per 1 mln of cells tested



Functional Studies - beta Thioredoxin reductase Assay Kit (ab83463)

Standard curve (colourimetric) : mean of duplicates (+/-SD) with background readings substracted



Functional Studies - Thioredoxin Reductase Assay Kit (ab83463)

Thioredoxin reductase Kinetic Data using ab83463.

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