Overview

**Product name**
Thioredoxin Reductase 1 (TXNRD1) Activity Assay Kit

**Detection method**
Colorimetric

**Precision**

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysates</td>
<td>4</td>
<td></td>
<td></td>
<td>2.9%</td>
</tr>
</tbody>
</table>

Intra-assay

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysates</td>
<td>4</td>
<td></td>
<td></td>
<td>3.2%</td>
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</tbody>
</table>

Inter-assay

**Sample type**
Cell culture extracts, Tissue Extracts

**Assay type**
Cell-based (quantitative)

**Range**
5 µg/ml - 1000 µg/ml

**Species reactivity**
- **Reacts with:** Human
- **Does not react with:** Mouse, Rat

**Product overview**
Abcam’s Thioredoxin Reductase 1 (TXNRD1) Activity Assay kit is designed for the sensitive and accurate measurement of Thioredoxin Reductase 1 activity in Human cell and tissue extracts. The assay uses a 96-well plate with an antibody specific to isoform 1 of thioredoxin reductase to isolate the isozyme pre-coated onto the wells. Samples are added to the wells and incubated at room temperature, any Thioredoxin Reductase 1 present in the sample will be immobilized in the well. After washing, the Reaction Buffer is added to each well and enzyme activity is measured. By analyzing the enzyme’s activity in an isolated context, outside of the cell and free from other isoforms, an accurate measurement of the enzyme’s functional state can be understood.

**Notes**
Thioredoxin Reductase 1 (TXNRD1) is a selenium-containing enzyme part of the thioredoxin system responsible for regulating oxidative stress and redox signaling via reduction of disulfide bonds. The dimer, Thioredoxin Reductase 1, reduces thioredoxin and other substrates using NADPH and an FAD cofactor. Thioredoxin Reductase 1 involvement in protecting against oxidative stress and injury, regulation of cellular development and growth, and various other
cellular processes make it an interesting target for studies of various cancers, AIDS, and other diseases. Humans express three different isozymes of thioredoxin reductase, isoform 1 is the cytosolic form, isoform 2 is the mitochondrial form, and isoform 3 is a testes specific form.

The enzyme activity is determined by following the NADPH-assisted reduction of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) to 5-thio-2-nitrobenzoic acid (TNB) which leads to increased absorbance at 412 nm. Two moles of TNB are formed from the oxidation of one mole of NADPH according to the reaction shown below.

\[ \text{DTNB} + \text{NADPH} + \text{H}^+ \leftrightarrow 2 \text{TNB} + \text{NADP}^+ \]

The molar extinction coefficient of TNB is \( 14.15 \times 10^3 \text{ mM}^{-1}\text{cm}^{-1} \). Thioredoxin Reductase 1 activity is controlled by enzyme amount. An antibody specific to isoform 1 of thioredoxin reductase is used to isolate the enzyme from a bulk protein source for isoform-specific enzymatic activity measurements. Because the activity measured is specific to the isolated enzyme, a thioredoxin reductase inhibitor is not required for background signal subtraction.

**Platform**
Microplate reader

**Properties**

**Storage instructions**
Store at +4°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>1 x 96 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Blocking Buffer</td>
<td>1 x 8ml</td>
</tr>
<tr>
<td>10X Buffer</td>
<td>1 x 25ml</td>
</tr>
<tr>
<td>200X DTNB</td>
<td>1 x 110µl</td>
</tr>
<tr>
<td>200X NADPH</td>
<td>1 vial</td>
</tr>
<tr>
<td>5X Activity Solution</td>
<td>1 x 5ml</td>
</tr>
<tr>
<td>Extraction Buffer (ab260490)</td>
<td>1 x 15ml</td>
</tr>
<tr>
<td>TXNRD1 96-well microplate</td>
<td>1 unit</td>
</tr>
</tbody>
</table>

**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**

**Images**
TXNRD1 Activity in Hela lysate with or without 20 µM aurothiomalate (ATM) as an inhibitor of TXNRD1 activity.

The data shown above was collected at the endpoint after 30 minutes.

After the rate/slope of each lane is extracted from the linear range of the time point data, it is expressed as rate (mOD/min) per microgram of cell lysate added per well. The extinction for the DTNB dye is 9.9/ mM / well.

The assay was used to determine the TXNRD1 activity in a series of normal cell lysates and tissue homogenates loaded at 250µg/mL.

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