Thioredoxin Reductase Assay Kit

Catalog No.: abx298808

Size: 100 Assays

Storage: Store the Assay Buffer at 4°C, the Inhibitor at 4°C in the dark, and the Substrate and Dye Reagent in the dark at -20°C.

Application: For quantitative detection of Thioredoxin Reductase activity in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, and other biological fluids.

Introduction: Thioredoxin Reductase is a common enzyme that catalyzes the reduction of thioredoxin by NADPH. This enzyme is involved through the thioredoxin system in cell growth, and protection against oxidative stress.

Thioredoxin Reductase catalyzes the reduction of the acid DTNB with NADPH. The reduced product, TNB, has a distinctive yellow colour, producing a maximum absorbance at 412 nm. The concentration of the reaction product is directly proportional to the enzyme activity, which can be measured by measuring the absorbance at 412 nm.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Inhibitor: 1 ml
4. Dye Reagent: 1 vial
5. Standard: 1 vial

Materials Required But Not Provided

1. Microplate reader (412 nm)
2. High-precision pipette and sterile pipette tips
3. Distilled water
4. Mortar
5. Centrifuge and centrifuge tubes
6. Timer
7. Ice

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 4 ml of Assay Buffer into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.

- **Dye Reagent Solution**
  Add 20 ml of Assay Buffer to the Dye Reagent vial and mix thoroughly. Ensure that the Dye Reagent has completely dissolved prior to use.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant, and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer, then allow to stand for 2 hours. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Serum and Plasma samples**
  Serum and plasma samples can be used directly.

B. Assay Procedure

Bring the Assay Buffer to room temperature prior to use.

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Set the sample and control wells and record the positions. Each sample should have at least one control. We recommend measuring each sample in duplicate.
2. Add 20 µl of each sample to the corresponding sample and control wells.
3. Add 60 µl of Assay Buffer to the sample wells.
4. Add 50 µl of Assay Buffer to the control wells.
5. Add 10 µl of Inhibitor to the control wells.
6. Add 20 µl of Substrate Solution to the sample and control wells.
7. Add 100 µl of Dye Reagent to the sample and control wells. Mix thoroughly. Start the timer.
8. At 10 seconds, read and record absorbance at 412 nm.
9. At 130 seconds, read and record absorbance at 412 nm.

C. Calculations

One unit of Thioredoxin Reductase activity is defined as the amount of enzyme required to reduce 1 nmol of DTNB per minute.

Thioredoxin Reductase activity per mg of protein:

$$\textbf{Thioredoxin Reductase}\ (\mathbf{U/mg}) = \frac{\mathbf{V_{Total}}}{\mathbf{V_{Sample}} \times \mathbf{T}} \times \frac{\left(\mathbf{OD_{Sample\ (130s)}} - \mathbf{OD_{Control\ (130s)}}\right) - \left(\mathbf{OD_{Sample(10s)}} - \mathbf{OD_{Control(10s)}}\right)}{\boldsymbol{\varepsilon} \times \boldsymbol{d} \times \mathbf{C_{Protein}}}$$

$$= \mathbf{612.7} \times \frac{\left(\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Control(130s)}}\right) - \left(\mathbf{OD_{Sample(10s)}} - \mathbf{OD_{Control(10s)}}\right)}{\mathbf{C_{Protein}}}$$

Thioredoxin Reductase activity per g of sample:

$$\textbf{Thioredoxin Reductase}\ (\mathbf{U/g}) = \frac{\mathbf{V_{Total}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{W} \times \mathbf{T}} \times \frac{\left(\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Control(130s)}}\right) - \left(\mathbf{OD_{Sample(10s)}} - \mathbf{OD_{Control(10s)}}\right)}{\boldsymbol{\varepsilon} \times \boldsymbol{d} \times \mathbf{W}}$$

$$= \mathbf{612.7} \times \frac{\left(\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Control(130s)}}\right) - \left(\mathbf{OD_{Sample(10s)}} - \mathbf{OD_{Control(10s)}}\right)}{\mathbf{W}}$$

Thioredoxin Reductase activity per 10^4 cells or bacteria:

$$\textbf{Thioredoxin Reductase}\ (\mathbf{U/10^4}) = \frac{\mathbf{V_{Total}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{W} \times \mathbf{T}} \times \frac{\left(\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Control(130s)}}\right) - \left(\mathbf{OD_{Sample(10s)}} - \mathbf{OD_{Control(10s)}}\right)}{\boldsymbol{\varepsilon} \times \boldsymbol{d} \times \mathbf{N}}$$

$$= \mathbf{612.7} \times \frac{\left(\mathbf{OD_{Sample(130s)}} - \mathbf{OD_{Control(130s)}}\right) - \left(\mathbf{OD_{Sample(10s)}} - \mathbf{OD_{Control(10s)}}\right)}{\mathbf{N}}$$

where:

$\mathbf{OD_{Sample(130s)}}$	The absorbance of the sample at 130s.
$\mathbf{OD_{Control(130s)}}$	The absorbance of the control at 130s.
$\mathbf{OD_{Sample(10s)}}$	The absorbance of the sample at 10s.
$\mathbf{OD_{Control(10s)}}$	The absorbance of the control at 10s.
$\boldsymbol{\varepsilon}$	Molar extinction coefficient (13.6 x 10^{-3} ml/nmol/cm)
$\boldsymbol{d}$	Optical path (0.6 cm)
$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{T}$	Reaction time (2 minutes)
$\mathbf{W}$	Weight of the sample (in g)
$\mathbf{N}$	Number of cells or bacteria (× 10^4)
$\mathbf{V_{Assay}}$	Volume of assay buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.02 ml)
$\mathbf{V_{Total}}$	Volume of standard (0.2 ml)