CREATIVE BIOMART®
Assay Kit

Thioredoxin Reductase Colorimetric Assay Kit

Product Information

Cat.No. Kit-0834

Product Overview

Thioredoxin Reductase Colorimetric Assay Kit provides a convenient method for detecting mammalian 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 405-414 nm. 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). The difference between the two results is the DTNB reduction due to TrxR activity.

Storage

This kit will perform as specified if stored at -20°C.

Kit Components

TrxR Assay Buffer (10X) 1 vial
Thioredoxin Reductase Control 1 vial
TrxR Inhibitor 2 vials
TrxR DTNB 1 vial
TrxR NADPH 2 vials
TrxR DMSO 1 vial
96-Well Plate (colorimetric assay) 1 plate
96-Well Cover Sheet 1 cover

Materials Required but Not Supplied

1. A plate reader capable of measuring absorbances between 405-414 nm.
2. Adjustable pipettes and a repeating pipettor.
3. A source of pure water; glass distilled water or HPLC-grade water is acceptable.

Technical Notes

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• It is recommended that an adjustable pipette be used to deliver reagents to the wells.
• 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.
• The final volume of the assay is 200 µl in all the wells.
• It is not necessary to use all the wells on the plate at one time.
• The assay temperature is 22°C.
• We recommend assaying samples in the presence and absence of ATM. Since several enzymes present in biological samples can reduce DTNB, ATM is used to determine the reduction of DTNB due only to TrxR activity.
• Twenty-three samples can be assayed in duplicate.
• Monitor the absorbance at 405-414 nm.

Preparation

Reagent Preparation
1. TrxR Assay Buffer (10X)
Dilute 3 ml of Assay Buffer concentrate with 27 ml of HPLC-grade water. This final Assay Buffer (50 mM potassium phosphate, pH 7.0, containing 50 mM potassium chloride, 1 mM EDTA, and 0.2 mg/ml BSA) should be used in the assay and for reconstituting the TrxR Inhibitor and NADPH. When stored at -20°C, this diluted Assay Buffer is stable for at least six months.
2. Thioredoxin Reductase (control)
The vial contains 100 µl of a solution of rat liver thioredoxin reductase (TrxR). The thawed enzyme should be stored on ice. The enzyme is ready to use as supplied.
3. TrxR Inhibitor
The vial contains a lyophilized powder of sodium aurothiomalate (ATM). Reconstitute the vial with 1 ml of diluted Assay Buffer before use. The reconstituted Inhibitor is stable for four hours. ATM is a specific thioredoxin inhibitor. Since several enzymes present in biological samples can reduce DTNB, the ATM is used to determine the reduction of DTNB specific to thioredoxin reductase. The concentration of Inhibitor used in the assay, 20 µM, will effectively remove all thioredoxin reductase activity.
4. TrxR DTNB

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Weigh 4 mg of DTNB into another vial, add 2 ml of dimethylsulfoxide (DMSO) and vortex until dissolved. Store the reagent at room temperature in the dark and use within four hours.
5. TrxR NADPH
The vial contains a lyophilized powder of NADPH. Reconstitute the vial with 2 ml of diluted Assay Buffer and store on ice. The reconstituted NADPH is stable for six hours.
6. TrxR DMSO
The vial contains 10 ml of DMSO. It is ready to use as supplied. Once thawed, DMSO can be stored at room temperature for six months.

Sample Preparation
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 5-10 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. Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the sample at -80°C. The sample will be stable for at least one month.

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 cells (1 x 10^8) by centrifugation (i.e., 1,000-2,000 x g for 10 minutes at 4°C). For adherent cells, do not harvest using proteolytic enzymes; rather use a rubber policeman.
2. Homogenize cell pellet in 5-10 ml of cold buffer (i.e., 50 mM potassium phosphate, pH 7.4, containing 1 mM EDTA).
3. Centrifuge at 10,000 x g for 15 minutes at 4°C.
4. Remove the supernatant for assay and store on ice. If not assaying on the same day, freeze the

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sample at -80°C. The sample will be stable for at least one month.

Assay Protocol

1. Background Wells - add 160 µl of diluted Assay Buffer to two wells.
2. Background + ATM Wells - add 140 µl of diluted Assay Buffer and 20 µl of ATM to two wells. NOTE: ATM does slightly react with DTNB so it is important to subtract this activity from the sample + ATM wells.
3. Positive Control Wells (rat liver TrxR) - add 140 µl of diluted Assay Buffer and 20 µl of rat liver TrxR (control) to two wells.
4. Sample Wells - add 140 µl of diluted Assay Buffer and 20 µl of sample to two wells.
5. Sample + ATM Wells - add 120 µl of diluted Assay Buffer, 20 µl of sample, and 20 µl of ATM to two wells.
6. Initiate the reactions by adding 20 µl of NADPH and 20 µl of DTNB to all the wells being used. Carefully shake the microtiter plate for 10 seconds to mix.
7. Read the absorbance once every minute at 405-414 nm using a plate reader to obtain at least five time points.

Analysis

Determination of the Reaction Rate

1. Determine the change in absorbance (ΔA405) per minute 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 - or-

b. Select two points on the linear portion of the curve and determine the change in absorbance during that time using the following equation:

ΔA405/min. =[A405 (Time 2) - A405 (Time 1)]/[Time 2 (min.) - Time 1 (min.)]

2. Determine the rate ΔA405/min. for the background and subtract this rate from all the wells, including the background + ATM, sample, sample + ATM, and positive control wells.
3. Use the following formulas to calculate the TrxR activity. The reaction rate at 405 or 414 nm can be determined using either the DTNB extinction coefficient of 7.92 mM-1 (405 nm) or 8.42 mM-1 (414 nm). The actual extinction coefficients for DTNB at 405 nm and 414 nm are 12.8 mM-1cm-1 and 13.6 mM-1cm-1, respectively. The values have been adjusted for the pathlength of the solution in the

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well (0.619 cm). One unit is defined as the NADPH-dependent production of 2 μmol of 2-nitro-5-thiobenzoate per minute at 22°C.

Sensitivity

Under the standardized conditions of the assay described in this booklet, the detection range of the assay is from 0.013-0.063 μmol/min/ml of TrxR activity, which is equivalent to an absorbance increase of 0.01-0.05 per minute.

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