CREATIVE BIOMART®
Assay Kit

NQO1 Activity Assay Kit

Product Information

Common Name

NQO1

Cat.No.

Kit-0633

Product Overview

NAD (P)H dehydrogenase [quinone] 1 (NQO1) Activity Assay kit is designed for the sensitive and accurate measurement of NQO1 activity in a number of sample types.NQO1 serves as a quinone reductase in connection with conjugation reactions of hydroquinones involved in detoxification pathways, NQO1 protects against quinone-induced damage by competing with potentially toxic one-electron pathways. It also functions in biosynthetic processes such as the vitamin K-dependent gamma-carboxylation of glutamate residues in prothrombin synthesis.The enzyme activity is determined by following the reduction of Menadione with cofactor NADH and the simultaneous reduction of WST1 which leads to increased absorbance at 440 nm.

Description

Peroxidases (EC number 1.11.1.x) are a large family of enzymes that typically catalyze a reaction of the form: ROOR'' + electron donor (2 e-) + 2H+ → ROH + R''OH. For many of these enzymes the optimal substrate is hydrogen peroxide, but others are more active with organic hydroperoxides such as lipid peroxides. Peroxidases can contain a heme cofactor in their active sites, or alternately redox-active cysteine or selenocysteine residues.

Storage

Store kit at 4°C immediately upon receipt.

Size

96 tests

Kit Components

Components 1 x 96 tests

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1000X Cofactor 1 vial
1000X Inhibitor 1 vial
100X Dye (lyophilized) 1 vial
100X Menadione 1 vial
100X NADH 1 vial
20X Basic Buffer 1 x 3ml
2X Extraction Buffer 1 x 15ml
96-well microplate 2 x 96-Wells

Materials Required but Not Supplied

Microplate reader capable of measuring absorbance at 440nm.
Method for determining protein concentration (BCA assay recommended).
Deionized water.
Multi and single channel pipettes.
Tubes for sample dilution.
Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors) and/or phosphatase inhibitors.

Target Species

Mouse, Rat, Cow, Human

Compatible Sample Types

Cell culture extracts, Adherent cells, Suspension cells, Tissue Extracts, Cell Lysate, Tissue Homogenate, Tissue Lysate

Preparation

REAGENT PREPARATION:
Equilibrate all reagents to room temperature (18-25°C) prior to use.
1. 1X Extraction Buffer
Prepare 1X Extraction Buffer by adding equal volumes of nanopure water and 2X Extraction Buffer. Mix gently and thoroughly. Unused 1X Extraction Buffers should be stored at 4°C.
2. Basic Buffer
Prepare Basic Buffer by adding 3 mL 20X Basic Buffer to 57 mL nanopure water. Mix gently and

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thoroughly.
3. 1,000X Cofactor
Resuspend the lyophilized 1,000X Cofactor by adding 100 μL nanopure water. Mix gently and thoroughly. After resuspension any unused 1,000X Cofactor should be stored at -20°C.
4. Supplemented Buffer
Prepare the Supplemented Buffer by adding 60 µL 1,000X Cofactor to 60 mL Basic Buffer. Mix gently and thoroughly. Any unused Supplemented should be stored at 4°C.
5. 100X NADH
Resuspend the 100X NADH by adding 1 mL nanopure water. Mix gently and thoroughly. After resuspension any unused 100X NADH should be stored at -20°C.
6. 100X Dye
Resuspend the lyophilized 100X Dye by adding 250 μL nanopure water. Mix gently and thoroughly. After resuspension any unused 100X Dye should be stored at -20°C.
7. 100X Menadione
Resuspend the lyophilized 100X Menadione by adding 200 μL nanopure water. Mix gently and thoroughly. After resuspension any unused 100X Menadione should be stored at -20°C.
8. 1,000X Inhibitor
Resuspend the lyophilized 1,000X Inhibitor by adding 200 μL nanopure water. Mix gently and thoroughly. After resuspension any unused 1,000X Inhibitor should be stored at -20°C.
Note: After resuspension, the inhibitor solution may appear cloudy. This is normal and will not affect the assay.
9. Reaction Buffer
Prepare the Reaction Buffer immediately prior to use. Prepare 500 μL Reaction Buffer for each 8 well strip used. Use the table
below for instructions on how to prepare the necessary volume of Reaction Buffer:
* Add the 100X Menadione to the buffer last.
Supplemented 100X NADH 100X Dye 100X Menadione* Total
Buffer (µL) (µL) (µL) (µL) (µL)
470 10 10 10 500
940 20 20 20 1,000

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1,410 30 30 30 1,500
1,880 40 40 40 2,000
2,350 50 50 50 2,500
2,820 60 60 60 3,000
3,290 70 70 70 3,500
3,760 80 80 80 4,000
4,230 90 90 90 4,500
4,700 100 100 100 5,000
5,170 110 110 110 5,500
5,640 120 120 120 6,000
6,110 130 130 130 6,500
6,580 140 140 140 7,000
7,050 150 150 150 7,500
7,520 160 160 160 8,000
7,990 170 170 170 8,500
8,460 180 180 180 9,000
8,930 190 190 190 9,500
9,400 200 200 200 10,000

10. Reaction Buffer + Inhibitor

Prepare the Reaction Buffer + Inhibitor solution immediately prior to use. Using the table below, mix the appropriate volume of Reaction Buffer (prepared in step 9.9) with inhibitor and mix gently but thoroughly.

Reaction Buffer (µL) 1,000X Inhibitor (µL)
250 0.5
500 1
750 1.5
1,000 2
1,250 2.5
1,500 3
1,750 3.5

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2,000 4
2,250 4.5
2,500 5
2,750 5.5
3,000 6
3,250 6.5
3,500 7
3,750 7.5
2,000 8
4,250 8.5
4,500 9
4,750 9.5
5,000 10

SAMPLE PREPARATION:
TYPICAL SAMPLE DYNAMIC RANGE -
Typical working ranges
Sample Type Range (µg/mL)
HepG2 Cell Lysate 0.5 - 100
Hela Cell Lysate 0.5 – 100
MCF7 Cell Lysate 0.5 – 100
3T3 Cell Lysate 0.5 – 200
Rat Heart Homogenate (RHH) 5 – 200
Bovine Heart Homogenate (BHH) 5 – 200
Note: Samples should be diluted to 2X the required final concentration.

1. Preparation of extracts from cell pellets
1.1 Collect non adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C.
1.2 Rinse cells twice with PBS.

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1.3 Solubilize cell pellet at 2x10^7 /mL in 1X Extraction Buffer.
1.4 Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.
1.5 Samples should be diluted to 2X the required final concentration in Supplemented Buffer.
2. Preparation of extracts from adherent cells by direct lysis (alternative protocol)
2.1 Remove growth media and rinse adherent cells 2 times in PBS.
2.2 Solubilize the cells by addition of Extraction Buffer directly to the plate (use 0.75 - 1.5 mL Extraction Buffer per confluent 15 cm diameter plate).
2.3 Scrape the cells into a test tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.
2.4 Samples should be diluted to 2X the required final concentration in Supplemented Buffer.
3. Preparation of extracts from tissue homogenates
3.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).
3.2 Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of the supplied extraction buffer. For lower amounts of tissue adjust volumes accordingly.
3.3 Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay.
3.4 Samples should be diluted to 2X the required final concentration in Supplemented Buffer.

PLATE PREPARATION
The 96 well plate included with this kit is supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
For each assay performed, a minimum of 2 wells must be used as the zero control.

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For each sample, a paired well should be used to test noninhibited activity and inhibited activity. For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Assay Protocol

Equilibrate all materials and prepared reagents to room temperature prior to use.
It is recommended to assay all controls and samples in duplicate.

1. Prepare all reagents as directed in section Preparation.
2. Samples should be diluted within the working range of the assay in Supplemented Buffer. Each sample needs to be loaded into paired wells, for the activity test with or without NQO1 Inhibitor.
3. Add 50 µL sample into each well.
4. Rapidly but carefully add 50 µL of Reaction Buffer + Inhibitor or Reaction Buffer to appropriate paired sample wells. Add Reaction Buffer + Inhibitor first.

Note: Change tips between different Reaction Buffers to prevent contamination of samples. Quickly remove any visible bubbles.

5. Immediately record the yellow color development with elapsed time in the microplate reader prepared with the following settings:

Mode: Kinetic
Wavelength: 440 nm
Time: up to 5 min.
Interval: 20 sec.
Shaking: Shake before and between readings
Alternative– In place of a kinetic reading, at a user defined, time record the endpoint OD data at 440 nm.

Analysis

NQO1 activity in each well is proportional to the increase in absorbance at 440 nm within each well. NQO1 activity was measured as Dicoumarol-sensitive activity. Therefore, NQO1 activity was calculated by subtracting the OD value with Inhibitor from the one without Inhibitor. The activity is expressed as the change in absorbance per minute per amount of sample loaded into the well. Examine the linear rate of increase in absorbance at 440 nm over time. Most microplate software is

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capable of performing this function.

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