ab184867 – NQO1 activity assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of NAD(P)H dehydrogenase [quinone] 1(NQO1) Activity in various samples.

This product is for research use only and is not intended for diagnostic use.

Storage temperature for this kit was updated 11 August 2018.

Protocol regarding reconstitution of the NADH component was updated on 16 May 2017. Please read all sections carefully before proceeding.

Last Updated 21 January 2019

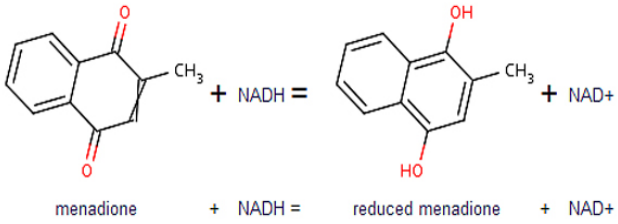
Table of Contents

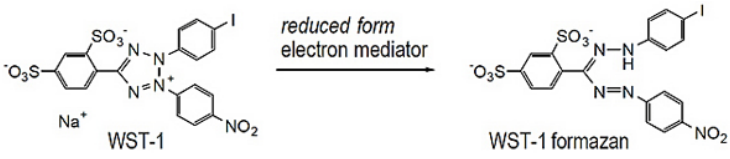
1. BACKGROUND

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.

Abcam's NAD(P)H dehydrogenase [quinone] 1(NQO1) Activity Assay kit is used to determine the activity of NQO1 in varies samples. 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.





The molar extinction coefficient for the dye is 37 X 10^3/M/cm. NQO1 activity is controlled by enzyme amount. Dicoumarol (3–3′-methylene-bis(4-hydroxycoumarin)) is a commonly used inhibitor of NQO1, which competes with NAD(P)H for binding to the oxidized form of NQO1. Dicoumarol thereby prevents reduction of various targets including Menadione, which used in this assay kit. NAD(P)H/WST1 combination also involve in other reductase activity, such as Complex I activity in OXPHOS, therefore, the NQO1 activity is presented as Dicoumarol-sensitive activity.

2. ASSAY SUMMARY

Prepare all reagents as described.

↓

Dilute samples to within a working range in Supplemented Buffer.

↓

Add samples to 96-well plate along with either Reaction Buffer or Reaction Buffer + Inhibitor

↓

Record the yellow color development at 440 nm

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC immediately upon receipt.

Refer to list of materials supplied (section 5) for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9&10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
20X Basic Buffer	3 mL	-20ºC
2X Extraction Buffer	15 mL	-20ºC
1000X Cofactor (Lyophilized)	1 vial	-20ºC
500X NADH (Lyophilized)	1 vial	-20ºC
100X Dye (Lyophilized)	1 vial	-20ºC
5000X Menadione (100%Ethanol)	100 µL	-20ºC
1000X Inhibitor (Lyophilized)	1 vial	-20ºC
Microplate	2 x 96-Wells	

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 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.

  NOTE: Inhibitors must be used when preparing extracts from tissue homogenates to prevent degradation of the target protein

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- A visualized color change right after adding the reaction buffer indicates fast reaction. Samples, therefore, should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 10).
- All samples should be mixed thoroughly and gently.
- Avoid multiply freeze/thaw of samples.
- When generating sample dilution series, it is advisable to change pipette tips after each step.
- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use.

9.1 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.

9.2 Basic Buffer

Prepare Basic Buffer by adding 3 mL 20X Basic Buffer to 57 mL nanopure water. Mix gently and thoroughly.

9.3 1000X 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.

9.4 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.

9.5 100X NADH

Resuspend the 500X NADH by adding 200 µL nanopure water (resuspended NADH concentration is 75mM). Further dilute the necessary amount of 500X NADH to 100X NADH with supplemented buffer right before use.

E.g. add 10µL 500XNADH to 40µL supplemented buffer to generate 50µL 100X NADH. Any unused 500X NADH should be stored at -20°C. Discard unused 100X NADH after the assay.

9.6 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.

9.7 **100X Menadione**

5000X Menadione is provided in 100% Ethanol. Keep vial on ice with the lid secured when not in use to prevent evaporation. Pipette 5µL 5000X Menadione into 245µL Supplemented Buffer. Mix solution well. This is 100X menadione solution. Unused 5000X Menadione should be stored at -20°C. Discard any unused 100X menadione after the assay.

9.8 **1000X 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.

9.9 **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 Buffer (µL)	100X NADH (µL)	100X Dye (µL)	100X Menadione* (µL)	Total (µL)
470	10	10	10	500
940	20	20	20	1,000
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

9.10 **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
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
4,000	8
4,250	8.5
4,500	9
4,750	9.5
5,000	10

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.

10.1 Preparation of extracts from cell pellets

10.1.1 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 4ºC.

10.1.2 Rinse cells twice with PBS.

10.1.3 Solubilize cell pellet at $2x10^7$/mL in 1X Extraction Buffer.

10.1.4 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.

10.1.5 Samples should be diluted to 2X the required final concentration in Supplemented Buffer.

10.2 **Preparation of extracts from adherent cells by direct lysis (alternative protocol)**

10.2.1 Remove growth media and rinse adherent cells 2 times in PBS.

10.2.2 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).

10.2.3 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.

10.2.4 Samples should be diluted to 2X the required final concentration in Supplemented Buffer.

10.3 **Preparation of extracts from tissue homogenates**

10.3.1 Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).

10.3.2 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.

10.3.3 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.

10.3.4 Samples should be diluted to 2X the required final concentration in Supplemented Buffer.

11. 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.
- For each sample, a paired well should be used to test non-inhibited activity and inhibited activity.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

12. ASSAY PROCEDURE

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

12.1 Prepare all reagents as directed in section 9 & 10.

12.2 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.

12.3 Add 50 µL sample into each well.

12.4 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.

12.5 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.

13. CALCULATIONS

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 capable of performing this function.

14. TYPICAL DATA

An example is shown below where the rate/slope is calculated between these time points.

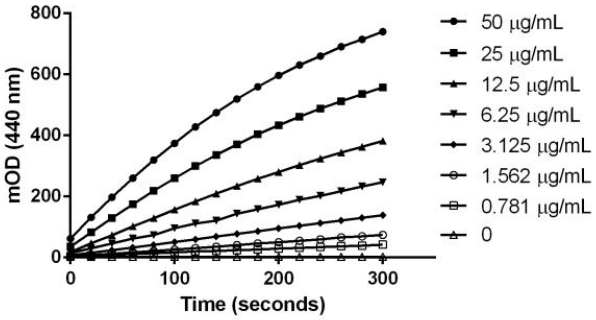


Figure 1. Raw data from various concentrations of HepG2 cell extracts.

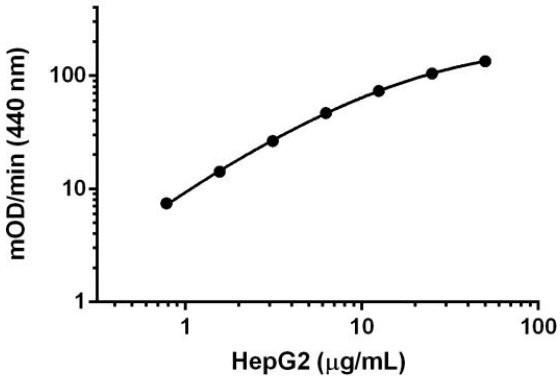
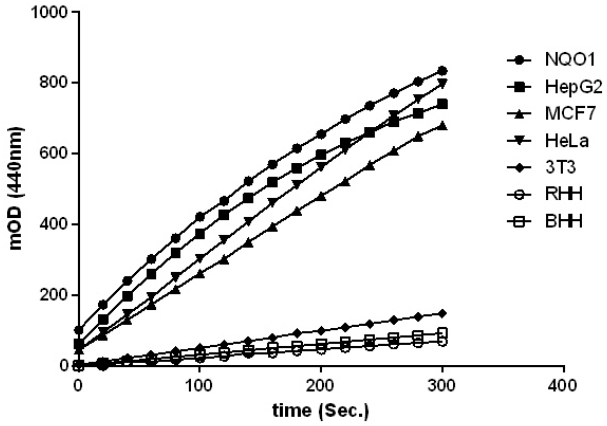


Figure 2: NQO1 Activity in HepG2 extract. This raw data is expressed as rate (mOD/min) per microgram of cell extract added per well as shown above. The molar extinction coefficient factor for the WST1 dye is 25.9/mM/well.

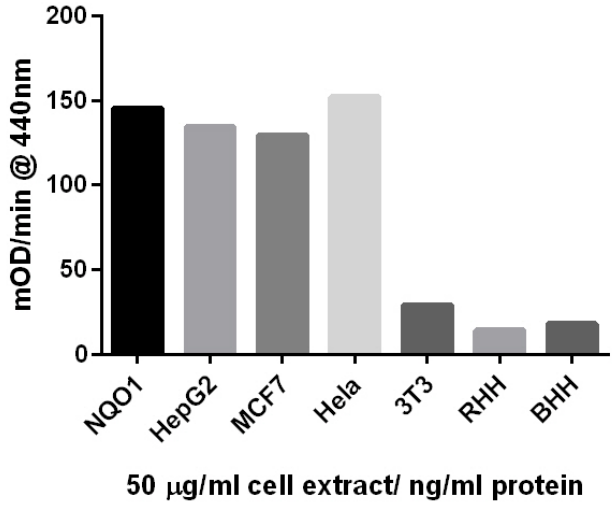
ASSAY REPRODUCIBILITY

	Intra-Assay	Inter-Assay
n=	3	3 X 3
%CV	2.183	6.035

3A


1000
800
600
400
200
0
mOD (440nm)
0
100
200
300
400
time (Sec.)
NQO1
HepG2
MCF7
HeLa
3T3
RHH
BHH

3B


200
150
100
50
0
mOD/min @ 440nm
NQO1
HepG2
MCF7
Hela
3T3
RHH
BHH
50 μg/ml cell extract/ ng/ml protein

3C

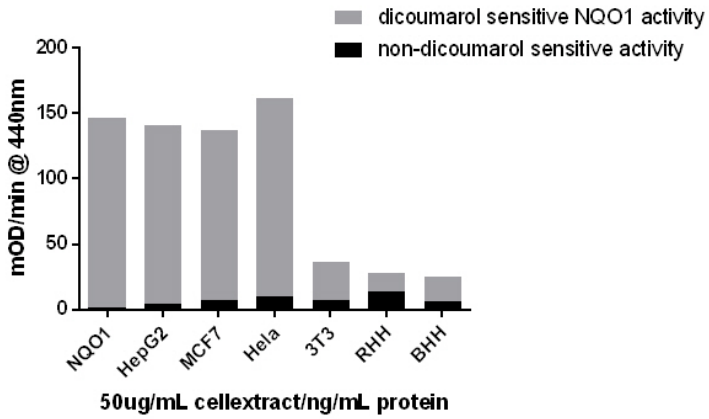


Figure 3. The assay was used to determine the Dicoumarol sensitive of NQO1 activity in a series of normal cell lysates and tissue homogenates. (**3A**) Raw data of NQO1 activity from 12.5 µg/mL of a series of normal cell lysate samples. (**3B**) Comparison of NQO1 activity in various sample types. (**3C**) Dicoumarol sensitive NQO1 activity/total activity in a series of normal cell lysates (50 µg/mL of each cell lysate).

15. ASSAY SPECIFICITY

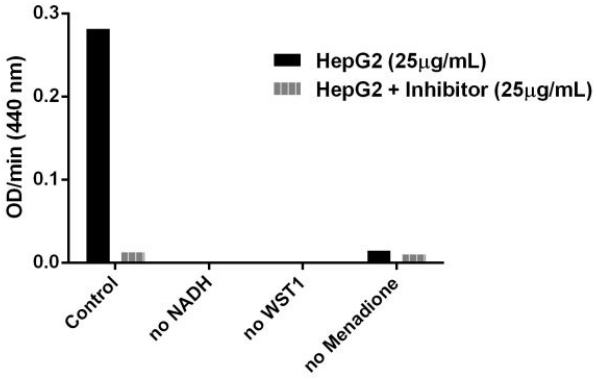


Figure 4. NQO1 activity assay components requirement test.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer needs to be at room temperature
	Component missed in the Reaction Buffer	Prepare fresh buffers and follow protocol exactly
Unexpected results	Plate read at incorrect wavelength	Use appropriate reader and filter settings described in datasheet
	Sample readings are outside linear range	Adjust concentrations of samples to be within the linear range of the assay
	Sample prepared in an unsuitable extraction reagent	Use the extraction buffer included in the kit to prepare lysates
Inconsistent readings	Sample has undergone too many freeze/ thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
	Kit components not fully thawed prior to beginning the assay	Wait for components to thaw completely and gently mix prior use
	Inaccurate Pipetting	Check pipettes
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

17. **NOTES**

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp