APL969Hu01 100μg
Active NADH Dehydrogenase, Quinone 1 (NQO1)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Val2~Lys274
Tags: N-terminal His-tag
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5%Trehalose .
Original Concentration: 200μg/mL
Applications: Cell culture; Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 8.9
Predicted Molecular Mass: 34.4kDa
Accurate Molecular Mass: 32kDa as determined by SDS-PAGE reducing conditions.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.
Store at 2-8ºC for one month.
Aliquot and store at -80ºC for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37ºC for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

VGRRALIVL AHSERTSFNY AMKEAAAAAL KKKGWEVVES DLYAMNFNPI
ISRKDITGKL KDPANFQYPA ESVLAYKEGH LSPDIVAEQK KLEAADLVIF
QFPLQWFGVP AILKGWFERV FIGEFAYTYA AMYDKGPFRS KKAVLSITTG
GSGSMYSLQG IHGDMNVILW PIQSGILHFC GFQVLEPQLT YSIGHTPADA
RIQILEGWKK RLENIWDETP LYFAPSSLFD LNFQAGFLMK KEVQDEEKNK
KFGLSVGHHL GKSIPTDNQI KARK

[ACTIVITY]

NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1), also known as DT-diaphorase, is a widely-distributed FAD-dependent flavoprotein that promotes 2-electron reductions of quinones, quinoneimines, nitroaromatics, and azo dyes. As a result it prevents the one electron reduction of quinones that results in the production of radical species. NQO1 is a highly-inducible enzyme that is regulated by the Keap1/Nrf2/ARE pathway. The increase and decrease of NQO1 levels are associated with decreased and increased susceptibilities to oxidative stress, respectively. Thus, NQO1 is a marker cytoprotective enzyme in oxidative stress. Independently of its catalytic function, NQO1 plays a role in regulating the proteosomal degradation of p53, p73a, and p33. NQO1 physically interacts with p53 and p73 in an NADH-dependent manner and protects them from 20S proteasomal degradation in a ubiquitin independent pathway. The activity assay of recombinant human NQO1 was measured by its ability to oxidize the substrate resazurin to resorufin. The rhNQO1 was diluted to 100 ug/ml in the assay buffer 50 mM HEPES, 0.2 M NaCl, 5 μM FAD, 0.05% Tween® 20, pH 7.5. 50 ul 100 ug/ml rhNQO1 was added into the microplate and start the reaction by adding 50 μl

substrate mixture of 400 uM beta-NADH and 20 uM resazurin which was diluted in assay buffer. Read at excitation and emission wavelengths of 540 nm and 585 nm (top read), respectively, in kinetic mode for 5 minutes. The specific activity of recombinant human NQO1 is >18 pmol/min/μg.

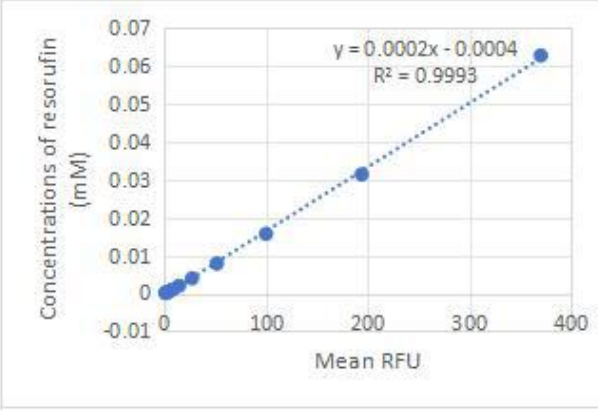

Figure 1. The standard curve of resorufin

RFU	resorufin (mM)
370.32992	0.0625
193.92992	0.03125
99.67992	0.015625
51.23992	0.0078125
26.69992	0.00390625
13.93992	0.001953125
7.57492	0.000976563
3.79692	0.000488281

One unit of enzyme activity is defined as the 1 μg of enzyme required to convert 1 pmol of resazurin in 1min.

Specific Activity (pmol/min/μg)= $\frac{\Delta OD * F}{T * N}$

△OD=Adjusted for Substrate Blank

F=Conversion Factor (convert from standard curve of resorufin)

T= Time

[IDENTIFICATION]

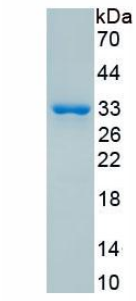

Figure 2. SDS-PAGE

Sample: Active recombinant NQO1, Human

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.