Anti-NQO1 Antibody [JF440-1]

ET1702-50

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IP, FC, IHC-P
Molecular Wt:	Predicted band size: 31 kDa
Clone number:	JF440-1

Description: NAD(P)H:quinone oxidoreductase 1 (NQO1) and NRH:quinone oxidoreductase (NQO2) are flavoproteins that catalyze the metabolic detoxification of quinones and their derivatives to hydroquinones, using either NADH or NADPH as the electron donor. This protects cells against quinone-induced oxidative stress, cytotoxicity, and mutagenicity. Many tumors overexpress NQO1, which is an obligate two-electron reductase that deactivates toxins and activates bioreductive anticancer drugs. NQO1, a 274 amino acid protein, is ubiquitously expressed, but the expression level varies among tissues. NQO1 gene expression is coordinately induced in response to xenobiotics, antioxidants, heavy metals and radiation. The antioxidant response element (ARE) in the NQO1 gene promoter is essential for expression and coordinated induction of NQO1. ARE activation by tert-butylhydroquinone is dependent on PI3-kinase, which lies upstream of Nrf2. Nrf2, c-Jun, Nrf1, Jun-B and Jun-D bind to the ARE and regulate expression and induction of NQO1 gene. Maf-Maf homodimers and possibly Maf-Nrf2 heterodimers play a role in negative regulation of ARE-mediated transcription, but Maf-Nrf1 heterodimers fail to bind with the NQO1 gene ARE and do not repress NQO1 transcription.

Immunogen:	Synthetic peptide within Human NQO1 aa 1-49 / 274.
Positive control:	HCT 116 cell lysate, HepG2 cell lysate, NIH/3T3 cell lysate, C2C12 cell lysate, Mouse kidney tissue lysate, Rat kidney tissue lysate, human breast cancer tissue, MCF7.
Subcellular location:	Cytoplasm.
Database links:	SwissProt: P15559 Human | Q64669 Mouse | P05982 Rat
Recommended Dilutions:	
WB	1:1,000-1:5,000
IP	Use at an assay dependent concentration.
FC	1:1,000
IHC-P	1:1,000
Storage Buffer:	1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.
Purity:	Protein A affinity purified.

Images

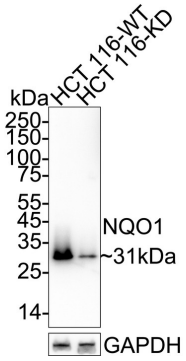

Fig1: Western blot analysis of NQO1 on different lysates with Rabbit anti-NQO1 antibody (ET1702-50) at 1/2,000 dilution.

Lane 1: HCT 116-si NT cell lysate
Lane 2: HCT 116-si NQO1 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 31 kDa
Observed band size: 31 kDa

Exposure time: 16 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1702-50) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

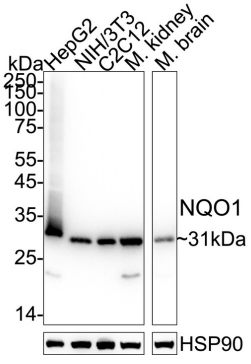

Fig2: Western blot analysis of NQO1 on different lysates with Rabbit anti-NQO1 antibody (ET1702-50) at 1/1,000 dilution.

Lane 1: HepG2 cell lysate (20 µg/Lane)
Lane 2: NIH/3T3 cell lysate (20 µg/Lane)
Lane 3: C2C12 cell lysate (20 µg/Lane)
Lane 4: Mouse kidney tissue lysate (40 µg/Lane)
Lane 5: Rat kidney tissue lysate (40 µg/Lane)

Predicted band size: 31 kDa
Observed band size: 31 kDa

Exposure time: 4 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1702-50) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

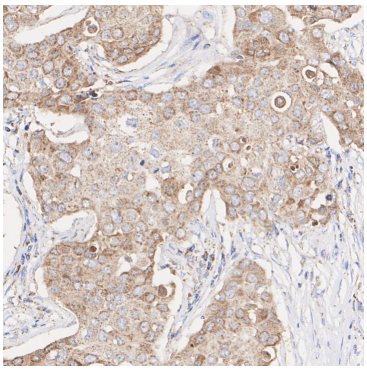

Fig3: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-NQO1 antibody (ET1702-50) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-50) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

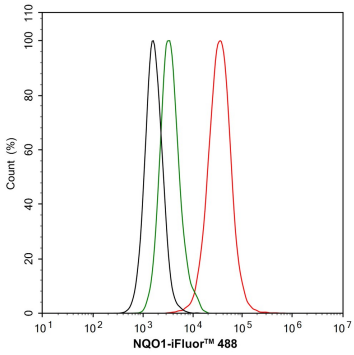

Fig4: Flow cytometric analysis of MCF7 cells labeling NQO1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1702-50, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Greco T et al. Ketogenic diet decreases oxidative stress and improves mitochondrial respiratory complex activity. J Cereb Blood Flow Metab 36:1603-13 (2016).
2. Kigoshi Y et al. CACUL1/CAC1 Regulates the Antioxidant Response by Stabilizing Nrf2. Sci Rep 5:12857 (2015).