# **Product Datasheet**

# Nrf2 Antibody - BSA Free NBP1-32822

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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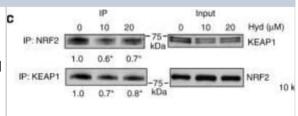
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Nrf2 Antibody - BSA Free	
Product Information	
Unit Size	0.1 ml
Concentration	Concentrations vary lot to lot. See vial label for concentration. If unlisted please contact technical services.
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.025% Proclin 300
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS, 20% Glycerol
Target Molecular Weight	68 kDa
Product Description	
Host	Rabbit
Gene ID	4780
Gene Symbol	NFE2L2
Species	Human, Mouse, Rat, Alligator, Avian, Plant, Zebrafish
Immunogen	Recombinant protein encompassing a sequence within the center region of human NRF2. The exact sequence is proprietary.
Product Application Details	
Applications	Western Blot, Simple Western, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation, Chromatin Immunoprecipitation (ChIP), Knockdown Validated
Recommended Dilutions	Western Blot 1:500-1:3000, Simple Western 1:20 - 1:500, Flow Cytometry Assay dependent, Immunohistochemistry 1:100-1:1000, Immunocytochemistry/ Immunofluorescence 1:100-1:1000, Immunoprecipitation 1:100-1:500, Immunohistochemistry-Paraffin 1:100-1:1000, Chromatin Immunoprecipitation (ChIP) Assay dependent, Knockdown Validated
Application Notes	In Simple Western internal validation: . See <u>Simple Western Antibody Database</u> for Simple Western validation: Rat skin wound at 0.5 mg/ml as sample; separated by size; antibody dilution of 1:20 - 1:500; observed molecular weight

## **Images**

Hydralazine enhances NRF2 signaling in SH-SY5Y cells. c Hydralazine reduced the interaction between NRF2 and KEAP1. Interactions were measured by reciprocal Co-IPs followed by western blot analysis. \*p < 0.05, two-tailed Student's t test, n = 3, mean +/- SD. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41467-017-02394-3), licensed under a CC-BY license.





was 78 kDa; detected by Chemiluminescence.

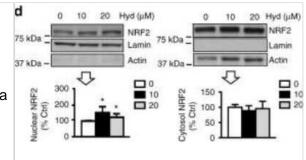
ChIP was performed with HepG2 chromatin extract and 5 ug of either normal rabbit IgG or anti-NRF2 antibody. The precipitated DNA was 30 detected by PCR with primer set targeting to GCLC gene locus. 25 ■GCLC gene locus **Fold enrichment** rabbit IgGi NRF2 antibody [N2C2], Internal Non-transfected (-) and NRF2-transfected (+, including 3xFlag-tag) 293T whole cell extracts (30ug) were separated by 5% SDS-PAGE, and the hNRF2 membrane was blotted with NRF2 antibody diluted by 1:1000. (kDa) 170 -130 -100 -55 -40 -RAW264.7 Untreated (-) and treated (+) RAW264.7 whole cell extracts (30 ug) were + LPS, 6 hr separated by 5% SDS-PAGE, and the membrane was blotted with NRF2 antibody [N2C2], Internal diluted at 1:500. The HRP-conjugated antirabbit IgG antibody (NBP2-19301) was used to detect the primary antibody. 250 -180 -130 -95 -Untreated (-) and treated (+) Rat-2 whole cell extracts (30 ug) were separated by 5% SDS-PAGE, and the membrane was blotted with NRF2 10 µM MG132, 16 hr antibody [N2C2], Internal (NBP1-32822) diluted at 1:500. The HRPconjugated anti-rabbit IgG antibody (NBP2-19301) was used to detect 250the primary antibody. 180-130-



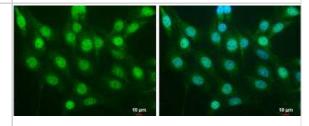
Untreated (-) and treated (+) Neuro2A whole cell extracts (30 ug) were separated by 5% SDS-PAGE, and the membrane was blotted with NRF2 10 µM MG132, 15 hi antibody [N2C2], Internal (NBP1-32822) diluted at 1:500. The HRPconjugated anti-rabbit IgG antibody (NBP2-19301) was used to detect the primary antibody. 258 - NRF2 Untreated (-) and treated (+) HepG2 whole cell extracts (30 ug) were separated by 5% SDS-PAGE, and the membrane was blotted with NRF2 10 pM MG132, 15 hr antibody [N2C2], Internal (NBP1-32822) diluted at 1:500. The HRPconjugated anti-rabbit IgG antibody (NBP2-19301) was used to detect the primary antibody. 250-- NRF2 95-Untreated (-) and treated (+) MDA-MB-231 nuclear extracts (30 ug) were MDA-MB-231 nuclear extract separated by 7.5% SDS-PAGE, and the membrane was blotted with 30 µM tBHQ, 4 hr NRF2 antibody [N2C2], Internal (NBP1-32822) diluted at 1:1000. (kDa) 180 72-43 -Untreated (-) and treated (+) HepG2 whole cell extracts (30 ug) were NBP1-51928 # Highly Cited Antibody separated by 5% SDS-PAGE, and the membranes were blotted with 10 µM MG132: 15 hr 10 µM MG132, 15 hr NRF2 antibody [N2C2], Internal (NBP1-32822) diluted at 1:500 and competitor's antibody diluted at 1:500. The HRP-conjugated anti-rabbit IgG antibody (NBP2-19301) was used to detect the primary antibody. 250-- NRF2



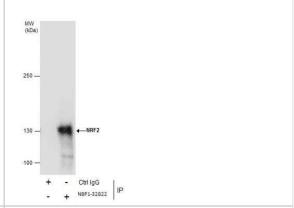
Hydralazine enhances NRF2 signaling in SH-SY5Y cells d NRF2 translocates to the nucleus with hydralazine treatment. Treated cells were subjected to cell fractionation and western blot analysis. \*p < 0.05 and \*\*p < 0.01, two-tailed Student's t test, n = 3, mean +/- SD. Image collected and cropped by CiteAb from the following publication (https://www.nature.com/articles/s41467-017-02394-3), licensed under a CC-BY license.



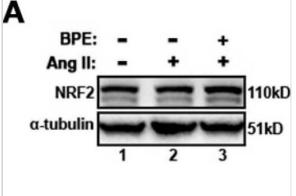
NIH/3T3 cells were fixed in 4% paraformaldehyde at RT for 15 min. Green: NRF2 protein stained by NRF2 antibody [N2C2], Internal diluted at 1:500. Blue: Hoechst 33342 staining. Scale bar = 10 um.



Immunoprecipitation of NRF2 protein from HepG2 whole cell extracts using 5 ug of NRF2 antibody [N2C2], Internal Western blot analysis was performed using NRF2 antibody [N2C2], Internal.. EasyBlot anti-Rabbit IgG was used as a secondary reagent.



Western Blot: Nrf2 Antibody [NBP1-32822] - Blueberry polyphenol extract (BPE) increases the expression of NRF2 & HO-1 while reducing NF-кB p65 phosphorylation in angiotensin (Ang) II-treated human aortic endothelial cells (HAECs). HAECs were treated with 200 µg/mL of BPE for 1 h then treated with 200 nM of Ang II for 12 h. Protein expression of NRF2 (A,B), HO-1 (C,D), & NF-кB p65 (E,F) were determined by Western blot. Quantification was performed using Image Lab (Bio-Rad Laboratories, Inc.). Data are expressed as mean  $\pm$  SD from nine (HO-1), & three (NRF2 & NF-кB) independent experiments. Values that do not share the same letter are significantly different from each other (p  $\leq$  0.05). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35453301), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Nrf2 Antibody [NBP1-32822] - NRF2 antibody validation. (A) NRF2 expression was silenced in MCF7 cells by transiently transfecting NRF2-specific siRNAs or a negative control siRNA for 48 h, then NRF2 protein expression was determined using two different antibodies (Abcam: ab31163; NOVUS: NBP1-32822). (B–D) 4T1 cells were treated with NRF2 activators, RA839 or tBHQ, or MG-132, a proteasome inhibitor, in the concentrations indicated for 48 h, then NRF2 protein expression was determined by western blotting using two different antibodies (Abcam: ab31163; NOVUS: NBP1-32822). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31461945), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

NRF2 silencing:

A

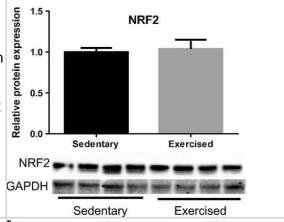
| SiNRF2 | SinRF2

Exercised

Western Blot: Nrf2 Antibody [NBP1-32822] - Maternal exercise during pregnancy on mitochondrial biogenesis in the fetal hearts. (A) Levels of relative mRNA expression measured by qRT $\square$ PCR. n = 9–12/group. Maternal exercise during pregnancy did not alter levels of mRNA in Ppargc1a & Tfam, while it significantly upregulated the levels of mRNA in Nrf1 & Nrf2. (B–D) Densitometric analyses of protein expression levels relative to the sedentary group with representative images of western blots were shown. No significant differences in PGC $\square$ 1 $\alpha$ , NRF1, & NRF2 (P > 0.05). n = 5–6/group. \* P < 0.05, significantly different from the sedentary group. Black bar: fetal hearts from sedentary dams; gray bar: fetal hearts from exercised dams. Image collected & cropped by CiteAb from the following publication

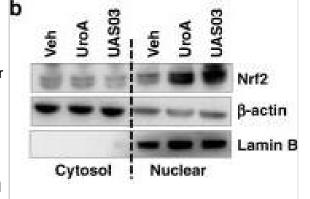
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Western Blot: Nrf2 Antibody [NBP1-32822] - Nrf2 is required for UroA/UAS03 mediated upregulation of tight junction proteins. a Nrf2 levels were determined by immunoblots in HT29 cells treated with vehicle/UroA/UAS03 (50 μM) for 24 h. b Nrf2 expression in cytosolic & nuclear fractions of HT29 cells treated with Veh/UroA/UAS03 (50 µM) for 6 h. c Immunofluorescence confocal images of HT29 cells treated with vehicle/UroA/UAS03 (50 μM) for 6 h. The cells were stained with anti-Nrf2 antibody & DAPI. Relative green fluorescence ( $n = \sim 20$  cells) intensity was measured. Scale bars indicate 25 µm. d Expression of Cldn4 & NQO1 in colon explants from WT, Nrf2-/-, & AhR-/- mice treated with vehicle/UroA/UAS03 (50 µM) for 24 h. Immunoblots were quantified using Image J software. e mRNA levels of Cldn4, Nrf2, & HO1 from colon explant cultures was measured by real-time PCR using SyBr green method. f C57BL/6, Nrf2-/-, & AhR-/- mice (n = 3) treated orally daily with veh or UroA/UAS03 (20 mg/kg) for 1 week. Cldn4 & NQO1 protein levels in colons were measured by immunoblots & quantified by Image J software. All in vitro studies were performed in triplicates. The immunoblots of colon explants & colon tissues were quantified from at least 6 independent runs. The levels of proteins were normalized to β-actin & Wild type vehicle treatment was set to 1 & calculated the fold changes. Statistics performed using unpaired t-test using Graphpad Prism software. Error bars, ±SEM; \*p < 0.05; \*\*p < 0.01; \*\*\*p < 0.001. Source Data are provided as a Source Data File Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/30626868), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Sedentary

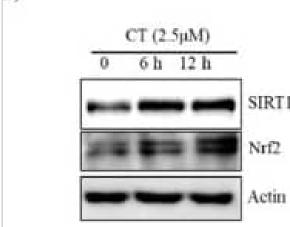
D



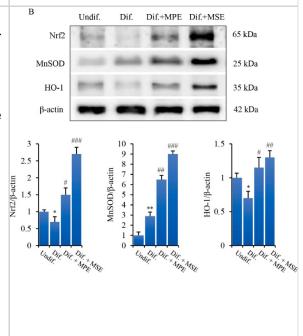
Western Blot: Nrf2 Antibody [NBP1-32822] - Modulation of Nrf2 & Keap1 🕻 mRNA & protein levels by compounds 1–6, curcumin (CURC), & dimethyl fumarate (DMF). (A-B) RNA from total cellular extracts of SH-SY5Y cells treated for 24 hours with 5 µM compounds or 20 µM DMF were analyzed for Nrf2 (A) & Keap1 (B) mRNA expression by RT-qPCR. GAPDH was used as housekeeping gene. Results are shown as mean ± SEM; no statistically significant data with Dunnett's multiple comparison test (A, n = 3, F ratio = 1.249; B, n = 3, F ratio = 1.671). (C–D) Cellular extracts of SH-SY5Y cells treated for 24 hours with compounds at 5 µM or 20 µM DMF were analyzed for Nrf2 (C) & Keap1 (D) protein levels by Western blot. Anti-tubulin was used as protein loading control. Results are shown as ratio (% of CTR) ± SEM; \*\*p < 0.01, versus CTR; Dunnett's multiple comparison test (C,  $n \ge 5$ , F ratio = 3.981; D, n = 3, F ratio = 0.4049). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32047434), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

110 KDa Nrf2
52 KDa Tubulin

Western Blot: Nrf2 Antibody [NBP1-32822] - CT activated AMPK/SIRT1 signaling. (A,B) HepG2 cells were treated with 2.5 µM CT for indicated times. Western blot analysis of phosphorylated AMPK, ACC, SIRT1, & Nrf2. (C) C57BL/6 mice were pair-fed either control or ethanol-containing diet with or without CT (20 or 40 mg/kg) for four weeks. Western blot analysis of phosphorylated AMPK, SIRT1. CYP2E1, & Nrf2. (D) HepG2 cells were incubated with 50 mM ethanol & treated with CT (2.5 or 5 µM) for 24 h. Western blot analysis of phosphorylated AMPK, SIRT1, CYP2E1, & Nrf2. (E) AML-12 cells were incubated with 50 mM ethanol & treated with CT (2.5 or 5 µM) for 24 h. Western blot analysis of phosphorylated AMPK & SIRT1. The images are representative (F) HepG2 cells were pretreated with CT (2.5 µM) for 3 h or with compound C (comp C) (10 µM) for 6 h, followed by ethanol (100 µM) treatment. Measurement of intracellular TG levels. Data are shown as mean ± SD of three independent experiments. #p < 0.05 vs. untreated control, \*\* p < 0.01 vs. ethanol-treated group. §§p < 0.01 vs. ethanol & CT-treated group. Densitometric analysis of western blots are given in Supplementary Figures S2 & S3A-G. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/31906014), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: Nrf2 Antibody [NBP1-32822] - MPE & MSE exert antioxidant effects in 3T3-L1 adipocytes. 3T3-L1 cells were treated with prodifferentiative agents for 8 days in the presence or absence of 100 µg/mL MPE or MSE, as reported in Methods. (A) Intracellular ROS were detected using the redox-sensitive fluorochrome H2-DCFDA. After differentiation, the medium was replaced with 10 µM H2DCFDA solution & the incubation was protracted for 30 min at 37 °C. The oxidation of the fluorochrome generates green fluorescence, which was visualized by a Leica microscope equipped with a DC300F camera using a FITC filter. Representative micrographs of fluorescence microscopy were taken at 200× magnification. (B). Western blotting analysis of Nrf2, MnSOD & HO-1 in 3T3-L1 cells differentiated without or with 100 µg/mL MPE or MSE. Equal loading of proteins was verified by immunoblotting for β-actin & showed values were assigned in relation to undifferentiated cells (Undif.). The bar graphs represent the mean of three independent experiments  $\pm$  SD. \* p < 0.05, \*\* p < 0.01 with respect to the undifferentiated 3T3-L1 cells, # p < 0.05, # p < 0.01 & # # p < 0.001with respect to the differentiated untreated 3T3-L1 cells (Dif.). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/35204243), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



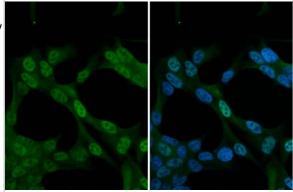


Western Blot: Nrf2 Antibody [NBP1-32822] - NRF2 modulates epirubicin C resistance in breast cancer cells. (A) MCF-7 & MCF-7 EpiR cells were treated for 24 h with epirubicin at 1 µM. MCF-7 & MCF-7 EpiR cells were stained with CellROX Deep Red reagent & analyzed for ROS levels by flow cytometry. Data were analyzed by FlowJo software. The mean fluorescence values were presented as relative ROS level compared to untreated cells (0 μM). Data presented as mean ± SD. Student's t-test was used to compare the means: \*\* p < 0.01; \*\*\* p < 0.001; n.s. nonsignificant. (N = 4) (B) Knockdown of NRF2 was achieved by transfecting 4 specific siRNA against NRF2 (siNRF2, 150pmol) to MCF-7 EpiR cells in a 6-well plate. Non-targeting siRNAs were used as control (NSC). At 24 h post-transfection, these cells were seeded in 6-well plates & treated with increasing doses of epirubicin for 14 days. Their sensitivity to epirubicin was assessed by clonogenic assay. Their clonogenicity in response to epirubicin was analyzed by two-way ANOVA & found to be significantly different (\*\* p < 0.01) from one another. (N = 3) (C) Expression of NRF2 in MCF-7 cells & MCF-7 EpiR cells was detected by Western blot.  $\beta$ -Tubulin served as the loading control. (N = 3). Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/32110852), licensed under a CC-BY license. Not internally tested by Novus Biologicals.

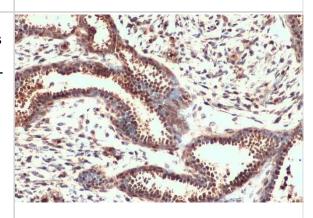
NRF2 — 95 kDa

β-Tubulin — 50 kDa

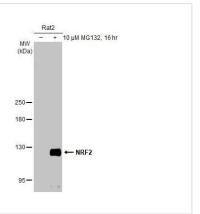
Immunocytochemistry/ Immunofluorescence: Nrf2 Antibody [NBP1-32822] - Nrf2 antibody [N2C2], Internal detects Nrf2 protein at nucleus by immunofluorescent analysis.Sample: Neuro2A cells were fixed in 4% paraformaldehyde at RT for 15 min.Green: Nrf2 stained by Nrf2 antibody [N2C2], Internal (NBP1-32822) diluted at 1:1000.



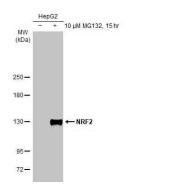
Immunohistochemistry-Paraffin: Nrf2 Antibody [NBP1-32822] - Nrf2 antibody [N2C2], Internal detects Nrf2 protein at cytoplasm and nucleus by immunohistochemical analysis. Sample: Paraffin-embedded human breast carcinoma. Nrf2 stained by Nrf2 antibody [N2C2], Internal (NBP1-32822) diluted at 1:500. Antigen Retrieval: Citrate buffer, pH 6.0, 15 min



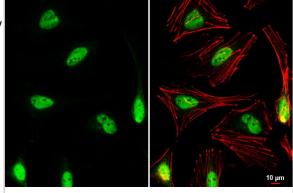
Western Blot: Nrf2 Antibody [NBP1-32822] - Untreated (-) and treated (+) Rat2 whole cell extracts (30 ug) were separated by 5% SDS-PAGE, and the membrane was blotted with Nrf2 antibody [N2C2], Internal (NBP1-32822) diluted at 1:2000. The HRP-conjugated anti-rabbit IgG antibody was used to detect the primary antibody, and the signal was developed with Trident ECL plus-Enhanced.



Western Blot: Nrf2 Antibody [NBP1-32822] - Untreated (-) and treated (+) HepG2 whole cell extracts (30 ug) were separated by 5% SDS-PAGE, and the membrane was blotted with Nrf2 antibody [N2C2], Internal (NBP1-32822) diluted at 1:2000. The HRP-conjugated anti-rabbit IgG antibody was used to detect the primary antibody.



Immunocytochemistry/ Immunofluorescence: Nrf2 Antibody [NBP1-32822] - Nrf2 antibody [N2C2], Internal detects Nrf2 protein at nucleus by immunofluorescent analysis.Sample: HeLa cells were fixed in 4% paraformaldehyde at RT for 15 min.Green: Nrf2 stained by Nrf2 antibody [N2C2], Internal (NBP1-32822) diluted at 1:1000.Red: phalloidin, a cytoskeleton marker, diluted at 1:200.Scale bar= 10 um.



#### **Publications**

Sheinin R, Salomon K, Yeini E et Al. interFLOW: maximum flow framework for the identification of factors mediating the signaling convergence of multiple receptors NPJ Syst Biol Appl 2024-06-10 [PMID: 38858414]

Angori S, Lakshminarayanan H, Banaei-Esfahani A et Al. Exploiting NRF2-ARE pathway activation in papillary renal cell carcinoma Int J Cancer 2024-12-20 [PMID: 39707614]

Rose KN, Zorlu M, Fassini A et Al. Neuroprotection of low dose carbon monoxide in Parkinson's disease models commensurate with the reduced risk of Parkinson's among smokers NPJ Parkinsons Dis 2024-08-22 [PMID: 39174550]

Albadrani GM, Altyar AE, Kensara OA et Al. Lycopene alleviates 5-fluorouracil-induced nephrotoxicity by modulating PPAR-?, Nrf2/HO-1, and NF-?B/TNF-?/IL-6 signals Ren Fail 2024-11-14 [PMID: 39540361]

Yan Huo, Abudureheman Mijiti, Ruonan Cai, Zhaohua Gao, Maierpu Aini, Abudukadier Mijiti, Zhaoling Wang, Rui Qie Scutellarin alleviates type 2 diabetes (HFD/low dose STZ)-induced cardiac injury through modulation of oxidative stress, inflammation, apoptosis and fibrosis in mice. Human & experimental toxicology 2022-03-07 [PMID: 34610774]

Ge, D;Chen, Q;Xie, X;Li, Q;Yang, Y; Unveiling the potent effect of vitamin D: harnessing Nrf2/HO-1 signaling pathways as molecular targets to alleviate urban particulate matter-induced asthma inflammation BMC pulmonary medicine 2024-01-25 [PMID: 38273268]

Shan Liu, Rui Zhang, Lan Zhang, Aige Yang, Yuqing Guo, Lei Jiang, Huijuan Wang, Shunjiang Xu, Huimin Zhou Oxidative stress suppresses PHB2 □mediated mitophagy in β□cells via the Nrf2/ PHB2 pathway Journal of Diabetes Investigation 2024-01-23 [PMID: 38260951]

Rudalska R, Harbig J, Snaebjornsson M et al. LXR alpha activation and Raf inhibition trigger lethal lipotoxicity in liver cancer Nature Cancer 2021-02-01 [PMID: 35122079]

Endo M, Tanaka Y, Fukuoka M et al. Wnt5a/Ror2 promotes Nrf2-mediated tissue protective function of astrocytes after brain injury Glia 2023-10-31 [PMID: 37904612]

Madi A, Sheinin R, Salomon K et al. interFLOW: maximum flow framework for the identification of factors mediating the signaling convergence of multiple receptors Research Square 2023-10-31 (IHC, Mouse)

Tabei Y, Abe H, Suzuki S et al. Sedanolide Activates KEAP1-NRF2 Pathway and Ameliorates Hydrogen Peroxide-Induced Apoptotic Cell Death International journal of molecular sciences 2023-11-20 [PMID: 38003720] (Simple Western, Human)

Details:

Dilution 1:50

Moniruzzaman M, Kumar S, Mukherjee M, Chakraborty SB Delineating involvement of MAPK/NF-?B pathway during mitigation of permethrin-induced oxidative damage in fish gills by melatonin Environmental toxicology and pharmacology 2023-11-13 [PMID: 37967690] (WB, Fish)

More publications at <a href="http://www.novusbio.com/NBP1-32822">http://www.novusbio.com/NBP1-32822</a>





# Novus Biologicals USA

10730 E. Briarwood Avenue Centennial, CO 80112

USA

Phone: 303.730.1950 Toll Free: 1.888.506.6887

Fax: 303.730.1966

nb-customerservice@bio-techne.com

#### **Bio-Techne Canada**

21 Canmotor Ave Toronto, ON M8Z 4E6

Canada

Phone: 905.827.6400 Toll Free: 855.668.8722 Fax: 905.827.6402

canada.inquires@bio-techne.com

#### **Bio-Techne Ltd**

19 Barton Lane Abingdon Science Park Abingdon, OX14 3NB, United Kingdom Phone: (44) (0) 1235 529449

Free Phone: 0800 37 34 15 Fax: (44) (0) 1235 533420 info.EMEA@bio-techne.com

### **General Contact Information**

www.novusbio.com

Technical Support: nb-technical@bio-

techne.com

Orders: nb-customerservice@bio-techne.com

General: novus@novusbio.com

## **Products Related to NBP1-32822**

HAF008 Goat anti-Rabbit IgG Secondary Antibody [HRP]

NB7160 Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]

NBP2-24891 Rabbit IgG Isotype Control

H00004780-P01-10ug Recombinant Human Nrf2 GST (N-Term) Protein

#### Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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