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SANTA CRUZ BIOTECHNOLOGY, INC.

Keap1 siRNA (h): sc-43878

BACKGROUND

Keap1 (Kelch-like ECH-associated protein 1, INrf2, KLHL19) is a stress sensing adaptor for the Cullin3 (Cul3)-dependent E3 ubiquitin ligase complex that negatively regulates Nrf2 (NF-E2-related factor 2). Steady state levels of proteins are under the influence of the ubiquitin pathway, which consists of ubiquitin activation (E1), conjugation (E2) and ligation (E3). Keap1 assembles into an E3 ubiquitin ligase complex with Cul3 and Rbx1 and targets lysine residues in the N-terminal Neh2 domain of Nrf2 for ubiquitin conjugation. The Keap1-Nrf2 system mediates cytoprotective gene expression in response to oxidative and/or electrophilic stresses. Keap1 constitutively suppresses Nrf2 activity under unstressed conditions, oxidants or electrophiles provoke the repression of Keap1 activity, inducing Nrf2 activation. Cys 273 and Cys 288 residues of Keap1 are required for suppressing Nrf2 nuclear accumulation. Keap1 sequesters Nrf2 in the cytoplasm through an active Crm1/exportin-dependent nuclear export mechanism.

REFERENCES

1. Zhang, D.D., et al. 2003. Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. Mol. Cell. Biol. 23: 8137-8151.
2. Kobayashi, A., et al. 2004. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. Mol. Cell. Biol. 24: 7130-7139.

CHROMOSOMAL LOCATION

Genetic locus: KEAP1 (human) mapping to 19p13.2.

PRODUCT

Keap1 siRNA (h) is a pool of 2 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Keap1 shRNA Plasmid (h): sc-43878-SH and Keap1 shRNA (h) Lentiviral Particles: sc-43878-V as alternate gene silencing products.

For independent verification of Keap1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-43878A and sc-43878B.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Keap1 siRNA (h) is recommended for the inhibition of Keap1 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

Keap1 (G-2): sc-365626 is recommended as a control antibody for monitoring of Keap1 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Keap1 gene expression knockdown using RT-PCR Primer: Keap1 (h)-PR: sc-43878-PR (20 µl, 554 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Ebert, B., et al. 2010. Regulation of human carbonyl reductase 3 (CBR3; SDR21C2) expression by Nrf2 in cultured cancer cells. Biochemistry 49: 8499-8511.
2. Ravuri, C., et al. 2013. Differential regulation of γ-glutamyltransferase and glutamate cysteine ligase expression after mitochondrial uncoupling: γ-glutamyltransferase is regulated in an Nrf2- and NFκB-independent manner. Free Radic. Res. 47: 394-403.
3. Zanotto-Filho, A., et al. 2016. Combined gene expression and RNAi screening to identify alkylation damage survival pathways from fly to human. PLoS ONE 11: e0153970.
4. Olagnier, D., et al. 2018. Nrf2 negatively regulates STING indicating a link between antiviral sensing and metabolic reprogramming. Nat. Commun. 9: 3506.
5. De Blasio, A., et al. 2019. A loop involving Nrf2, miR-29b-1-5p and Akt, regulates cell fate of MDA-MB-231 triple-negative breast cancer cells. J. Cell. Physiol. 235: 629-637.
6. Yu, J., et al. 2019. Overexpression of miR-200a-3p promoted inflammation in sepsis-induced brain injury through ROS-induced NLRP3. Int. J. Mol. Med. 44: 1811-1823.
7. Roos, N.J., et al. 2020. The uricosuric benzbromarone disturbs the mitochondrial redox homeostasis and activates the Nrf2 signaling pathway in Hep G2 cells. Free Radic. Biol. Med. 152: 216-226.

RESEARCH USE

For research use only, not for use in diagnostic procedures.