

Keap1 (h2): 293T Lysate: sc-171655

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.
3. Zhang, D.D., et al. 2004. Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. *Mol. Cell. Biol.* 24: 10941-10953.
4. Devling, T.W., et al. 2005. Utility of siRNA against Keap1 as a strategy to stimulate a cancer chemopreventive phenotype. *Proc. Natl. Acad. Sci. USA* 102: 7280-7285A.
5. Velichkova, M., et al. 2005. Keap1 regulates the oxidation-sensitive shuttling of Nrf2 into and out of the nucleus via a CRM1-dependent nuclear export mechanism. *Mol. Cell. Biol.* 25: 4501-4513.
6. Hosoya, T., et al. 2005. Differential responses of the Nrf2-Keap1 system to laminar and oscillatory shear stresses in endothelial cells. *J. Biol. Chem.* 280: 27244-27250.
7. Hong, F., et al. 2005. Specific patterns of electrophile adduction trigger Keap1 ubiquitination and Nrf2 activation. *J. Biol. Chem.* 280: 31768-31775.
8. Nguyen, T., et al. 2005. Nrf2 controls constitutive and inducible expression of ARE-driven genes through a dynamic pathway involving nucleocytoplasmic shuttling by Keap1. *J. Biol. Chem.* 280: 32485-32492.

CHROMOSOMAL LOCATION

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

PRODUCT

Keap1 (h2): 293T Lysate represents a lysate of human Keap1 transfected 293T cells and is provided as 100 µg protein in 200 µl SDS-PAGE buffer.

APPLICATIONS

Keap1 (h2): 293T Lysate is suitable as a Western Blotting positive control for human reactive Keap1 antibodies. Recommended use: 10-20 µl per lane.

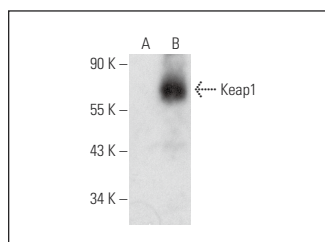
Control 293T Lysate: sc-117752 is available as a Western Blotting negative control lysate derived from non-transfected 293T cells.

Keap1 (A-4): sc-515432 is recommended as a positive control antibody for Western Blot analysis of enhanced human Keap1 expression in Keap1 transfected 293T cells (starting dilution 1:100, dilution range 1:100-1:1,000).

RECOMMENDED SUPPORT REAGENTS

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048.

DATA



Keap1 (A-4): sc-515432. Western blot analysis of Keap1 expression in non-transfected: sc-117752 (A) and human Keap1 transfected: sc-171655 (B) 293T whole cell lysates.

STORAGE

Store at -20° C. Repeated freezing and thawing should be minimized. Sample vial should be boiled once prior to use. Non-hazardous. No MSDS required.

RESEARCH USE

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

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.