Store at -20°C

#48768

p62/KEAP1/NRF2 Pathway Sampler Kit

1 Kit (7 x 20 µl)

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

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New 02/20

For Research Use Only. Not For Use In Diagnostic Procedures.

Product Includes	Product #	Quantity	Mol. Wt.	Isotype/Source
SQSTM1/p62 (D1Q5S) Rabbit mAb	39749	20 µl	62 kDa	Rabbit IgG
Phospho-SQSTM1/p62 (Ser349) (E7M1A) Rabbit mAb	16177	20 µl	62 kDa	Rabbit IgG
LC3A/B (D3U4C) XP® Rabbit mAb	12741	20 µl	14, 16 kDa	Rabbit IgG
KEAP1 (D6B12) Rabbit mAb	8047	20 µl	60-64 kDa	Rabbit IgG
NRF2 (D1Z9C) XP® Rabbit mAb	12721	20 µl	97-100 kDa	Rabbit IgG
HO-1 (E3F4S) Rabbit mAb	43966	20 µl	28 kDa	Rabbit IgG
NQO1 (D6H3A) Rabbit mAb	62262	20 µl	29 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

Description: The p62/KEAP1/NRF2 Pathway Sampler Kit provides an economical means of detecting the non-canonical mechanism of NRF2 activation involving autophagy. The kit includes enough antibodies to perform two western blot experiments with each primary antibody.

Background: The cap 'n' collar (CNC), leucine zipper (bZIP) transcription factor NRF2 (also called nuclear factor erythroid 2-related factor 2 (NFE2L2)) is the master regulator of the cellular antioxidant response, regulating the expression of over 200 genes that contain antioxidant response elements (AREs) in their regulatory regions by heterodimerizing with small MAF proteins (1). While NRF2 is expressed in all cell types, its basal protein levels are usually kept low during homeostatic conditions, mainly by KEAP1 (Kelch-like ECH-associated protein 1). Under normal conditions, KEAP1 binds to and targets NRF2 for ubiquitination-dependent proteasomal degradation. Upon oxidative stress, KEAP1 is modified on some sensor cysteines, affecting its conformation and thus interfering its binding to NRF2, allowing newly synthesized NRF2 to accumulate and translocate to the nucleus to activate its target genes, including HO-1 (heme oxygenase 1) and NQO1 (NAD(P)H:quinone oxidoreductase 1) (2,3). Another mode of NRF2 regulation involves the autophagy adapter protein p62 (or sequestosome 1 [SQSTM1]) in a KEAP1-dependent but cysteine-independent manner, the so called non-canonical pathway. Autophagy is a tightly regulated cellular quality control system that removes damaged proteins or organelles. Autophagy can also be activated to degrade macromolecules to provide nutrients under cellular starvation stress. p62, especially upon phosphorylation at Ser349 (Ser351 in mouse p62), can compete with NRF2 for binding KEAP1 and, as a result, p62 sequesters KEAP1 into the autophagosome and prevents KEAP1-mediated NRF2 degradation. This process may also require autophagy protein LC3. In addition, studies also found that KEAP1 is a p62-regulated substrate for autophagy-mediated degradation, indicating that p62 also plays a role in controlling KEAP1 turnover (4,5). Dysregulation of autophagy results in prolonged NRF2 activation and this may contribute to many diseases, including cancer and neurodegenerative diseases (6-9).

Specificity/Sensitivity: Each antibody in the p62/KEAP1/NRF2 Pathway Sampler Kit detects endogenous levels of its target protein. Phospho-SQSTM1/p62 (Ser349) (E7M1A) Rabbit mAb recognizes endogenous levels of SQSTM1/p62 protein only when phosphorylated at Ser349.

Source/Purification: Monoclonal antibody is produced by immunizing animals with synthetic peptides corresponding to residues surrounding Leu44 of human LC3B (conserved in LC3A), Ala275 of human NRF2, Leu228 of human NQO1, Leu118 of mouse HO-1, and near the carboxy termini of human KEAP1 and SQSTM1/p62 proteins. Phosphorylation-specific monoclonal antibodies are produced by immunizing rabbits with synthetic phosphopeptides corresponding to Ser349 of human SQSTM1/p62 protein.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Background References:

(1) Zhu, M. and Fahl, W.E. (2001) *Biochem Biophys Res Commun* 289, 212-9.

(2) Bellezza, I. et al. (2018) *Biochim Biophys Acta Mol Cell Res* 1865, 721-33.

(3) Yamamoto, M. et al. (2018) *Physiol Rev* 98, 1169-203.

(4) Jiang, T. et al. (2015) *Free Radic Biol Med* 88, 199-204.

(5) Nezis, I.P. and Stenmark, H. (2012) *Antioxid Redox Signal* 17, 786-93.

(6) Rojo de la Vega, M. et al. (2018) *Cancer Cell* 34, 21-43.

(7) Sánchez-Martín, P. et al. (2019) *FEBS J* 286, 8-23.

(8) Shah, S.Z.A. et al. (2018) *Front Mol Neurosci* 11, 310.

(9) Ichimura, Y. and Komatsu, M. (2018) *Front Oncol* 8, 210.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.