Recombinant Human His10-KEAP1/CUL3/NEDD8/RBX1

Catalog Number: E3-701

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived human KEAP1/CUL3/NEDD8/RBX1 protein Accession # Q14145, Q13618, Q15843, P62877
Predicted Molecular Mass	70 kDa (KEAP1), 101 kDa (neddylated CUL3), 12 kDa (RBX1)

SPECIFICATIONS

Activity	Typical protein concentration for use in vitro will depend on experimental conditions. For in vitro ubiquitination reactions, we suggest an initial KEAP1/CUL3(NEDD8)/RBX1 concentration between 10-50 nM.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain.
Formulation	X mg/ml (X µM) in 50 mM HEPES pH 7.5, 200 mM NaCl, 10% (v/v) Glycerol, 1 mM DTT See Certificate of Analysis for details.

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 48 months from date of receipt, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

Kelch-like ECH-associated protein (KEAP1) is the substrate recognition subunit of a BCR (BTB-CUL3-RBX) E3 ubiquitin ligase complex. KEAP1 contains an N-terminal BB Domain that mediates interaction with CUL3, a central BACK Domain that plays a role in protein-protein interactions, and six C-terminal Kelch Domains that function in substrate recognition. In the BCR complex, CUL3 serves as a scaffold that organizes one or more BTB (BR-C, Ttk and Bab, also known as a POZ Domain) substrate recognition subunits with the RBX subunit and contributes to catalysis through positioning of the substrate and an E2 ubiquitin-conjugating enzyme. KEAP1 is an important sensor of oxidative and electrophilic stress. Normally the KEAP1/CUL3/RBX1 complex ubiquitinates and causes degradation of NRF2, a transcription factor regulating expression of many cytoprotective genes. Oxidative stress results in the inability of KEAP1 to ubiquitinate NRF2, leading to the latter proteins accumulation and subsequent upregulation of genes involved in cellular oxidative stress responses. This recombinant complex consists of an N-terminal 10-His tagged Cullin-3 (UniProt Q13618), untagged RBX1 (UniProt P62877) and untagged KEAP1 (UniProt Q14145-1). Cullin-3 is neddylated (Q15843) in this complex.

References:

1. Eggler, A.L. et al. (2009) Biochem. J. doi:10.1042/BJ20090471
2. Eggler, A.L. et al. (2005) Proc. Natl. Acad. Sci. doi: 10.1073/pnas.0502402102
3. Mills, E.L. et al. (2018) Nature doi: 10.1038/nature25986
4. Wei, J. et al. (2021) JACS doi: 10.1021/jacs.1c04841

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