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CRL4/CRBN Targeted Protein Degradation Complex Antibody Sampler Kit

#72032

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

Product Includes	Product #	Quantity	Mol. Wt.	Isotype/Source
CRBN (D8H3S) Rabbit mAb	71810	20 µL	55 kDa	Rabbit IgG
CUL4A Antibody	2699	20 µL	80, 82 kDa	Rabbit
DDB-1 (D4C8) Rabbit mAb	6998	20 µL	127 kDa	Rabbit IgG
RBX1 (D3J5I) Rabbit mAb	11922	20 µL	13 kDa	Rabbit IgG
NEDD8 (19E3) Rabbit mAb	2754	20 µL	9 kDa	Rabbit IgG
Ubiquitin (E4I2J) Rabbit mAb	43124	20 µL	9-300 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 CRL4/CRBN Targeted Protein Degradation Complex Antibody Sampler Kit provides an economical means of detecting the individual components of a CRL4/CRBN E3 ubiquitin ligase complex, including free and conjugated forms of both NEDD8 and ubiquitin. The kit includes enough antibodies to perform two western blot experiments with each primary antibody.

Background: Targeted protein degradation is an experimental method of drug-based protein targeting that leverages endogenous E3 ubiquitin ligases and the ubiquitin proteasome system (UPS) to selectively degrade target proteins. It is being actively explored as a therapeutic strategy to target and degrade specific proteins that contribute to disease progression (1). This approach differs from traditional small-molecule therapeutics that seek to suppress disease proteins (e.g., kinases) by sterically blocking catalytic domains. Protein-targeting chimeras (PROTACs) are the prototypical protein "degraders". PROTACs are bivalent chemical ligands that induce proximity between a target protein and an E3 ubiquitin ligase, resulting in ubiquitination of the target protein, and its subsequent degradation by the UPS (2,3). Cereblon (CRBN) is the substrate-recognition component of a Cullin-RING-ubiquitin ligase complex (CRL4/CRBN) that was among the first to be recognized for its therapeutic potential via targeted protein degradation (4). The CRL4/CRBN complex is comprised of CRBN, DDB-1, RBX1, and the scaffold protein CUL4A; its ligase activity is dynamically regulated via the covalent modification (neddylation) of CUL4A by NEDD8 (5). In unrelated mechanistic studies of multiple myeloma drugs, it was revealed that phthalimides (e.g., thalidomide, lenalidomide) promoted CRBN-dependent recruitment, ubiquitination, and proteasomal degradation of the immunological transcription factors Ikaros (IKZF1) and Aiolos (IKZF3) (6). The discovery that phthalimides were functioning as PROTACs, by eliciting the selective degradation of what were previously considered "undruggable" protein targets, led to a rapid acceleration and expansion of research into targeted protein degradation, with the promise of novel therapies for diseases deemed largely intractable using conventional small-molecule therapies (7-9).

Specificity/Sensitivity: Each antibody in the CRL4/CRBN Targeted Protein Degradation Complex Antibody Sampler Kit detects endogenous levels of its target protein. NEDD8 (19E3) Rabbit mAb detects endogenous levels of both free and conjugated NEDD8 protein. The antibody does not cross-react with other ubiquitin family members, including ubiquitin, SUMO-1, SUMO-2, SUMO-3, and ISG15. Ubiquitin (E4I2J) Rabbit mAb recognizes endogenous levels of free ubiquitin and polyubiquitinated proteins. This antibody is able to detect free ubiquitin, linear polyubiquitin (M1-linked), and homotypic polyubiquitin chains consisting of K6, K11, K27, K29, K33, K48, and K63 linkages.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Pro44 of human CRBN protein, Gly832 of human DDB-1 protein, Gly35 of human ubiquitin protein, the amino terminus of human NEDD8 protein, and the carboxy terminus of human RBX1 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser12 of human CUL4A. Antibodies are purified by peptide affinity chromatography.

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) Bond, M.J. and Crews, C.M. (2021) *RSC Chem Biol* 2, 725-742.

(2) Sakamoto, K.M. et al. (2001) *Proc Natl Acad Sci* U S A 98, 8554-9.

(3) Sakamoto, K.M. et al. (2003) *Mol Cell Proteomics* 2, 1350-8.

(4) Krönke, J. et al. (2014) *Science* 343, 301-5.

(5) Hofmann, H. et al. (2013) *J Virol* 87, 11741-50.

(6) Lu, G. et al. (2014) *Science* 343, 305-9.

(7) Shirasaki, R. et al. (2021) *Cell Rep* 34, 108532.

(8) Alabi, S.B. and Crews, C.M. (2021) *J Biol Chem* 296, 100647.

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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.