SANTA CRUZ BIOTECHNOLOGY, INC.

UBE2D (F-6): sc-166277

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The Power to Question

BACKGROUND

Ubiquitin is an abundant, highly conserved protein found in all eukaryotic cells either free or covalently attached to cellular proteins. The primary function of ubiquitin in mammalian systems is to clear abnormal, foreign and improperly folded proteins by targeting them for proteosome degradation. UBE2D proteins, including UBE2D1 (ubiquitin-conjugating enzyme E2D1 or UBC5A), UBE2D2 (ubiquitin-conjugating enzyme E2D2 or UBC5B) and UBE2D3 (ubiquitin-conjugating enzyme E2D3 or UBC5C) are E2 ubiquitin-conjugating enzymes that catalyze the ubiquitination of IκBα in a phosphorylation and SCFB-TRCP-dependent manner. Specifically, E1 first transfers a ubiquitin residue to the E2 component (a UBE2D protein) and the UBE2D protein then associates with an E3 ubiquitin-protein ligase, which immediately transfers that residue to a protein that is targeted for degradation. In this fashion, the ubiquitin targets the IκBα for degradation by a proteasome thus lifting the inhibitory effect of IκBα on NFκB and allowing NFκB to enter the nucleus.

REFERENCES

1. Ciechanover, A. 1994. The ubiquitin-proteasome proteolytic pathway. Cell 79: 13-21.
2. Ciechanover, A., et al. 1994. The ubiquitin-mediated proteolytic pathway: mechanisms of recognition of the proteolytic substrate and involvement in the degradation of native cellular proteins. FASEB J. 8: 182-191.
3. Hochstrasser, M. 1995. Ubiquitin, proteasomes and the regulation of intracellular protein degradation. Curr. Opin. Cell Biol. 7: 215-223.
4. Liakopoulos, D., et al. 1998. A novel protein modification pathway related to the ubiquitin system. EMBO J. 17: 2208-2214.
5. Schwarz, S.E., et al. 1998. The ubiquitin-like proteins SMT3 and SUMO-1 are conjugated by the UBC9 E2 enzyme. Proc. Natl. Acad. Sci. USA 95: 560-564.
6. Gong, L., et al. 1999. Identification of the activating and conjugating enzymes of the NEDD8 conjugation pathway. J. Biol. Chem. 274: 12036-12042.
7. Raboy, B., et al. 1999. Heat-induced cell cycle arrest of *Saccharomyces cerevisiae:* involvement of the RAD6/UBC2 and WSC2 genes in its reversal. Mol. Microbiol. 32: 729-739.
8. Strack, P., et al. 2000. SCFB-TRCP and phosphorylation dependent ubiquitination of IκBα catalyzed by UBC3 and UBC4. Oncogene 19: 3529-3536.

SOURCE

UBE2D (F-6) is a mouse monoclonal antibody specific for an epitope mapping between amino acids 111-143 at the C-terminus of UBE2D of human origin.

PRODUCT

Each vial contains 200 µg IgG_1 kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-166277 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% stabilizer protein).

APPLICATIONS

UBE2D (F-6) is recommended for detection of reactive with all isoforms of UBE2D of mouse, rat and human origin by Western Blotting (starting dilution 1:100, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

UBE2D (F-6) is also recommended for detection of reactive with all isoforms of UBE2D in additional species, including canine, bovine, porcine and avian.

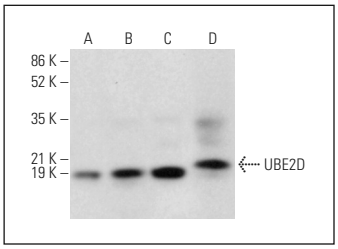
Molecular Weight of UBE2D: 17 kDa.

Positive Controls: F9 cell lysate: sc-2245, HeLa whole cell lysate: sc-2200 or NIH/3T3 whole cell lysate: sc-2210.

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. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

DATA



UBE2D (F-6): sc-166277. Western blot analysis of UBE2D expression in HeLa (**A**), NIH/3T3 (**B**) and F9 (**C**) whole cell lysates and mouse testis tissue extract (**D**). Detection reagent used: m-IgGκ BP-HRP: sc-516102.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. 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.