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SANTA CRUZ BIOTECHNOLOGY, INC.

UBE2D1 siRNA (m): sc-41680

BACKGROUND

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, components of the protein ubiquitination pathway. Protein ubiquitination covalent modification targets proteins for 26 S proteasome-dependent degradation. Three classes of enzymes influence the conjugation mechanism of ubiquitin to protein. E1 ubiquitin-activating enzymes mediate ATP-dependent charging of ubiquitin via formation of a high energy thiol ester bond between the C-terminus of ubiquitin and a cysteine within itself. Thiol ester-linked ubiquitin is then transferred from E1 to a cysteine residue in an E2 ubiquitin-conjugating enzyme. E2 enzymes in conjunction with E3 ubiquitin-protein ligases transfer ubiquitin monomers and polyubiquitin chains to the substrate target protein, where stable isopeptide linkages are formed.

REFERENCES

1. Schwarz, S.E., et al. 1998. Characterization of human hect domain family members and their interaction with UbcH5 and UbcH7. J. Biol. Chem. 273: 12148-12154.
2. Gehrke, S.G., et al. 2003. UbcH5A, a member of human E2 ubiquitin-conjugating enzymes, is closely related to SFT, a stimulator of iron transport, and is up-regulated in hereditary hemochromatosis. Blood 101: 3288-3293.
3. Gu, H., et al. 2003. The degradation of promyelocytic leukemia and Sp100 proteins by herpes simplex virus 1 is mediated by the ubiquitin-conjugating enzyme UbcH5a. Proc. Natl. Acad. Sci. USA 100: 8963-8968.
4. Dominguez, C., et al. 2004. Structural model of the UbcH5B/CNOT4 complex revealed by combining NMR, mutagenesis, and docking approaches. Structure 12: 633-644.
5. Knutson, M., et al. 2004. Developmental, regional, and cellular expression of SFT/UbcH5A and DMT1 mRNA in brain. J. Neurosci. Res. 76: 633-641.
6. Saville, M.K., et al. 2004. Regulation of p53 by the ubiquitin-conjugating enzymes UbcH5B/C *in vivo*. J. Biol. Chem. 279: 42169-42181.
7. Houben, K., et al. 2004. Solution structure of the ubiquitin-conjugating enzyme UbcH5B. J. Mol. Biol. 344: 513-526.

CHROMOSOMAL LOCATION

Genetic locus: Ube2d1 (mouse) mapping to 10 B5.3.

PRODUCT

UBE2D1 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see UBE2D1 shRNA Plasmid (m): sc-41680-SH and UBE2D1 shRNA (m) Lentiviral Particles: sc-41680-V as alternate gene silencing products.

For independent verification of UBE2D1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41680A, sc-41680B and sc-41680C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

UBE2D1 siRNA (m) is recommended for the inhibition of UBE2D1 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

UBE2D (C-6): sc-166278 is recommended as a control antibody for monitoring of UBE2D1 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

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) 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor UBE2D1 gene expression knockdown using RT-PCR Primer: UBE2D1 (m)-PR: sc-41680-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.