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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic



UBE2F siRNA (m): sc-154852

BACKGROUND

Ubiquitination is an important mechanism through which three classes of enzymes act in concert to target short-lived or abnormal proteins for destruction. The three classes of enzymes involved in ubiquitination are the ubiquitin-activating enzymes (E1s), the ubiquitin-conjugating enzymes (E2s) and the ubiquitin-protein ligases (E3s). UBE2F (ubiquitin-conjugating enzyme E2F), also known as NCE2, is a 185 amino acid protein that belongs to the E2 family of ubiquitin conjugating enzymes. UBE2F functions to accept the ubiquitin-like protein NEDD8 from an E1 complex and to catalyze the ATP-dependent attachment of NEDD8 to other proteins, playing a role in the pathway of protein degradation. The gene encoding UBE2F maps to chromosome 2q37.3, which encodes over 1,400 genes and comprises nearly 8% of the human genome.

REFERENCES

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4. Podust, V.N., Brownell, J.E., Gladysheva, T.B., Luo, R.S., Wang, C., Coggins, M.B., Pierce, J.W., Lightcap, E.S. and Chau, V. 2000. A Nedd8 conjugation pathway is essential for proteolytic targeting of p27^{Kip1} by ubiquitination. Proc. Natl. Acad. Sci. USA 97: 4579-4584.
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CHROMOSOMAL LOCATION

Genetic locus: Ube2f (mouse) mapping to 1 D.

PRODUCT

UBE2F 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 UBE2F shRNA Plasmid (m): sc-154852-SH and UBE2F shRNA (m) Lentiviral Particles: sc-154852-V as alternate gene silencing products.

For independent verification of UBE2F (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-154852A, sc-154852B and sc-154852C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

UBE2F siRNA (m) is recommended for the inhibition of UBE2F 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

UBE2F (C-11): sc-398668 is recommended as a control antibody for monitoring of UBE2F 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-IgG_k BP-HRP: sc-516102 or m-IgG_k 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-IgG_k BP-FITC: sc-516140 or m-IgG_k 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 UBE2F gene expression knockdown using RT-PCR Primer: UBE2F (m)-PR: sc-154852-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.