SANTA CRUZ BIOTECHNOLOGY, INC.

UDG siRNA (h): sc-37803

BACKGROUND

When misincorporation or cytosine deamination positions the RNA nucleotide uracil into DNA, uracil-DNA gycosylase (UDG) excises the uracil via a repair enzymatic pathway. This is done by cleaving the N-C1' gylcosylic bond between the base and deoxyribose, in both single and double-stranded DNA. While initiating the first steps of DNA repair, UDG undergoes a conformational change from the "open" unbound state to the "closed" DNA-bound state, creating a catalytic center. The bound UDG effectively flips the uridine nucleotide into the catalyic center and cleaves the glycosylic bond to excise the uracil. The open-to-closed conformation change is centered on a B zipper in the UDG. UDG alters the orientation electron orbitals to favor electron transpositions, thus taking advantage of conformational strain to catapult the cleavage of the gylcosylic bond. Two isoforms of UDG, UDG1 and UDG1A, have been characterized. The UDG1 isoform localizes to the mitochondria. UDG1A is a processed isoform containing a unique 44 residue amino-terminus which localizes this isoform to the nucleus.

REFERENCES

1. Aasland, R., et al. 1990. Chromosomal assignment of human uracil-DNA glycosylase to chromosome 12. Genomics 7: 139-141.
2. Slupphaug, G., et al. 1995. Properties of a recombinant human uracil-DNA glycosylase from the UNG gene and evidence that UNG encodes the major uracil-DNA glycosylase. Biochemistry 34: 128-138.
3. Slupphaug, G., et al. 1996. A nucleotide-flipping mechanism from the structure of human uracil-DNA glycosylase bound to DNA. Nature 384: 87-92.
4. Parikh, S.S., et al. 1997. Base excision repair enzyme family portrait: integrating the structure and chemistry of an entire DNA repair pathway. Structure 5: 1543-1550.
5. Parikh, S.S., et al. 1998. Base excision repair initiation revealed by crystal structures and binding kinetics of human uracil-DNA glycosylase with DNA. EMBO J. 17: 5214-5226.
6. Lindahl, T., et al. 1999. Quality control by DNA repair. Science 286: 1897-1905.

CHROMOSOMAL LOCATION

Genetic locus: UNG (human) mapping to 12q24.11.

PRODUCT

UDG siRNA (h) 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 UDG shRNA Plasmid (h): sc-37803-SH and UDG shRNA (h) Lentiviral Particles: sc-37803-V as alternate gene silencing products.

For independent verification of UDG (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-37803A, sc-37803B and sc-37803C.

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

UDG siRNA (h) is recommended for the inhibition of UDG expression in human 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

UDG (B-7): sc-390255 is recommended as a control antibody for monitoring of UDG 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 UDG gene expression knockdown using RT-PCR Primer: UDG (h)-PR: sc-37803-PR (20 µl, 446 bp). 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.