SANTA CRUZ BIOTECHNOLOGY, INC.

DDB1 siRNA (h): sc-37797

BACKGROUND

Damaged DNA binding protein (DDB) is a heterodimer composed of two subunits, p127 and p48, which are designated DDB1 and DDB2, respectively. The DDB heterodimer is involved in repairing DNA damaged by ultraviolet light. Specifically, DDB, also designated UV-damaged DNA binding protein (UV-DDB), xeroderma pigmentosum group E binding factor (XPE-BF) and hepatitis B virus X-associated protein 1 (XAP-1), binds to damaged cyclobutane pyrimidine dimers (CPDs). Mutations in the DDB2 gene are implicated as causes of xeroderma pigmentosum group E, an autosomal recessive disease in which patients are defective in nucleotide excision DNA repair. XPE is characterized by hypersensitivity of the skin to sunlight with a high frequency of skin cancer as well as neurologic abnormalities. The hepatitis B virus (HBV) X protein interacts with DDB1, which may mediate HBx transactivation.

REFERENCES

1. Dualan, R., et al. 1995. Chromosomal localization and cDNA cloning of the genes (DDB1 and DDB2) for the p127 and p48 subunits of a human damage-specific DNA binding protein. Genomics 29: 62-69.
2. Nichols, A.F., et al. 1996. Mutations specific to the xeroderma pigmentosum group E Ddb$^-$ phenotype. J. Biol. Chem. 271: 24317-2420.
3. Stohr, H., et al. 1998. Refined mapping of the gene encoding the p127 kDa UV-damaged DNA-binding protein (DDB1) within 11q12-q13.1 and its exclusion in Best's vitelliform macular dystrophy. Eur. J. Hum. Genet. 6: 400-405.
4. Lin, G.Y., et al. 1998. The V protein of the paramyxovirus SV5 interacts with damage-specific DNA binding protein. Virology 249: 189-200.
5. Nichols, A.F., et al. 2000. Human damage-specific DNA-binding protein p48. Characterization of XPE mutations and regulation following UV irradiation. J. Biol. Chem. 275: 21422-21428.
6. Zolezzi, F., et al. 2000. Studies of the murine DDB1 and DDB2 genes. Gene 245: 151-219.
7. Amundson, S.A., et al. 2000. Identification of potential mRNA biomarkers in peripheral blood lymphocytes for human exposure to ionizing radiation. Radiat. Res. 154: 342-346.

CHROMOSOMAL LOCATION

Genetic locus: DDB1 (human) mapping to 11q12.2.

PRODUCT

DDB1 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 DDB1 shRNA Plasmid (h): sc-37797-SH and DDB1 shRNA (h) Lentiviral Particles: sc-37797-V as alternate gene silencing products.

For independent verification of DDB1 (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-37797A, sc-37797B and sc-37797C.

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

DDB1 siRNA (h) is recommended for the inhibition of DDB1 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

DDB1 (E-11): sc-376860 is recommended as a control antibody for monitoring of DDB1 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 DDB1 gene expression knockdown using RT-PCR Primer: DDB1 (h)-PR: sc-37797-PR (20 µl, 585 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.