SANTA CRUZ BIOTECHNOLOGY, INC.

TFIIH p89 siRNA (h): sc-36655

BACKGROUND

Initiation of transcription from protein-coding genes in eukaryotes is a complex process that requires RNA polymerase II, as well as families of basal transcription factors. Binding of the factor TFIID (TBP) to the TATA box is believed to be the first step in the formation of a multiprotein complex containing several additional factors, including TFIIA, TFIIB, TFIIE, TFIIF and TFIIH. TFIIH (or BTF2) is a multisubunit transcription/DNA repair factor that possesses several enzymatic activities. The core of TFIIH is composed of five subunits, designated p89 (XPB or ERCC3), p62, p52, p44 and p34. Additional subunits of the TFIIH complex are p80 (XPD or ERCC2) and the ternary kinase complex composed of Cdk7, cyclin H and MAT1. Both p89 and p80 have ATP-dependent helicase activity. The p62, p52 and p44 subunits have been shown to be involved in nucleotide excision repair.

REFERENCES

1. Conaway, R.C., et al. 1989. An RNA polymerase II transcription factor has an associated DNA-dependent ATPase (dATPase) activity strongly stimulated by the TATA region of promoters. Proc. Natl. Acad. Sci. USA 86: 7356-7360.
2. Weeda, G., et al. 1990. A presumed DNA helicase encoded by ERCC3 is involved in the human repair disorders xeroderma pigmentosum and Cockayne's syndrome. Cell 62: 777-791.
3. Weber, C.A., et al. 1990. ERCC2: cDNA cloning and molecular characterization of a human nucleotide excision repair gene with high homology to yeast RAD3. EMBO J. 9: 1437-1447.
4. Fischer, L., et al. 1991. Cloning of the 62-kilodalton component of basic transcription factor BTF2. Science 257: 1392-1395.

CHROMOSOMAL LOCATION

Genetic locus: ERCC3 (human) mapping to 2q14.3.

PRODUCT

TFIIH p89 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 TFIIH p89 shRNA Plasmid (h): sc-36655-SH and TFIIH p89 shRNA (h) Lentiviral Particles: sc-36655-V as alternate gene silencing products.

For independent verification of TFIIH p89 (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-36655A, sc-36655B and sc-36655C.

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

TFIIH p89 siRNA (h) is recommended for the inhibition of TFIIH p89 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

TFIIH p89 (G-10): sc-271500 is recommended as a control antibody for monitoring of TFIIH p89 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 TFIIH p89 gene expression knockdown using RT-PCR Primer: TFIIH p89 (h)-PR: sc-36655-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Yi, J.M., et al. 2016. Triptolide induces cell Killing in multidrug-resistant tumor cells via CDK7/RPB1 rather than XPB or p44. Mol. Cancer Ther. 15: 1495-1503.
2. Kemp, M.G. 2017. DNA damage-induced ATM- and Rad-3-related (ATR) kinase activation in non-replicating cells is regulated by the XPB subunit of transcription factor IIH (TFIIH). J. Biol. Chem. 292: 12424-12435.

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.