SANTA CRUZ BIOTECHNOLOGY, INC.

BTF3a/b siRNA (m): sc-38514

BACKGROUND

The initiation of gene transcription involves the ordered assembly of a multi-protein complex on proximal promoter elements such as the TATA box. In addition to RNA polymerase II, the transcription factors class II (TFII) family of proteins are required for initiation of transcription, as the first step in the formation of this initiation complex is the stable binding of TFIID to the TATA box. An additional TFII related protein, BTF3, does not directly associate with the proximal promoter, but rather forms a stable complex with RNA pol II and facilitates RNA pol II assembling into the complex. The BTF3 gene is ubiquitously expressed and encodes for two protein isoforms, BTF3a and BTF3b, which are produced from alternative splicing. The BTF3 proteins are identical except that BTF3b lacks the first 44 amino acids at the N-terminal of BTF3a. As a consequence of this deletion, BTF3b is unable to induce transcription, despite being able to bind RNA pol II. Additionally, BTF3a and BTF3b associate with the widely expressed protein kinase CK2. CK2 phosphorylates BTF3a, as well as TFIIB, and is required for the efficient transcription of the tRNA and 55 rRNA genes by RNA pol III.

REFERENCES

1. Zheng, X.M., et al. 1987. A general transcription factor forms a stable complex with RNA polymerase B (II). Cell 50: 361-368.
2. Zheng, X.M., et al. 1990. Sequencing and expression of complementary DNA for the general transcription factor BTF3. Nature 344: 556-559.
3. Kanno, M., et al. 1992. Genomic structure of the putative BTF3 transcription factor. Gene 117: 219-228.
4. Moncollin, V., et al. 1992. Class II (B) general transcription factor (TFIIB) that binds to the template-committed preinitiation complex is different from general transcription factor BTF3. Proc. Natl. Acad. Sci. USA 89: 397-401.
5. Parvin, J.D., et al. 1994. Multiple sets of basal factors initiate transcription by RNA polymerase II. J. Biol. Chem. 269: 18414-18421.
6. George, C.P., et al. 1995. A spectrum of mechanisms for the assembly of the RNA polymerase II transcription preinitiation complex. Mol. Cell. Biol. 15: 1049-1059.
7. Grein, S., et al. 1999. BTF3 is a potential new substrate of protein kinase CK2. Mol. Cell. Biochem. 191: 121-128.

CHROMOSOMAL LOCATION

Genetic locus: Btf3 (mouse) mapping to 13 D1.

PRODUCT

BTF3a/b 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 BTF3a/b shRNA Plasmid (m): sc-38514-SH and BTF3a/b shRNA (m) Lentiviral Particles: sc-38514-V as alternate gene silencing products.

For independent verification of BTF3a/b (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-38514A, sc-38514B and sc-38514C.

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

BTF3a/b siRNA (m) is recommended for the inhibition of BTF3a/b 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

BTF3a/b (A-5): sc-166093 is recommended as a control antibody for monitoring of BTF3a/b 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 BTF3a/b gene expression knockdown using RT-PCR Primer: BTF3a/b (m)-PR: sc-38514-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.