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TFIIA- γ shRNA (h2) Lentiviral Particles: sc-44145-V

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, TFIIIE, TFIIF and TFIIH. Recognition of the TATA binding element by TBP, one of the first steps in transcription initiation, may be regulated by TFIIA. TFIIA consists of three subunits designated TFIIA- α , TFIIA- β and TFIIA- γ , and it interacts with both TBP and a TAF (TBP-associated factor). It has been demonstrated that the basic region of TBP is essential for TFIIA-dependent function of TBP.

REFERENCES

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3. 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.
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CHROMOSOMAL LOCATION

Genetic locus: GTF2A2 (human) mapping to 15q22.2.

PRODUCT

TFIIA- γ shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see TFIIA- γ siRNA (h2): sc-44145 and TFIIA- γ shRNA Plasmid (h2): sc-44145-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

TFIIA- γ shRNA (h2) Lentiviral Particles is recommended for the inhibition of TFIIA- γ expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

TFIIA- γ (D-6): sc-374483 is recommended as a control antibody for monitoring of TFIIA- γ 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TFIIA- γ gene expression knockdown using RT-PCR Primer: TFIIA- γ (h2)-PR: sc-44145-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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