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E6-AP shRNA (h) Lentiviral Particles: sc-43742-V

BACKGROUND

E6-associating protein (E6-AP), also designated ubiquitin protein ligase E3A (UBE3A), is a component of the ubiquitin-mediated proteolytic pathway that selectively targets proteins for degradation by the 26S proteasome. Ubiquitin (Ub) is directly conjugated to protein substrates by the transfer of Ub from an E2 ubiquitin conjugating enzyme to the target protein. This conjugation is facilitated by the enzymatic activity of E3 ubiquitin ligase family members such as E6-AP. Several substrates of E6-AP have been identified and include the tumor suppressor protein p53 and the mammalian homolog of Rad23, HHR23A. Previous studies have indicated that E6-AP associates with the human papillomavirus E6 oncogene, which forms a complex with p53 and thereby potentiates E6-AP mediated ubiquitination of p53. Genetic mutations that impair E6-AP activity result in the accumulation of p53 in the cytoplasm, and in many instances, these mutations are associated with the development of the rare neurodevelopmental disorder Angelman syndrome (AS), which is characterized by severe motor dysfunction and mental retardation.

REFERENCES

- Jentsch, S. 1992. The ubiquitin-conjugation system. *Annu. Rev. Genet.* 26: 179-207.
- Huibregtse, J.M., et al. 1993. Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. *Mol. Cell. Biol.* 13: 775-784.
- Haas, A.L., et al. 1997. Pathways of ubiquitin conjugation. *FASEB J.* 11: 1257-1268.
- Yamamoto, Y., et al. 1997. The human E6-AP gene (UBE3A) encodes three potential protein isoforms generated by differential splicing. *Genomics* 41: 263-266.

CHROMOSOMAL LOCATION

Genetic locus: UBE3A (human) mapping to 15q11.2.

PRODUCT

E6-AP shRNA (h) 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 E6-AP siRNA (h): sc-43742 and E6-AP shRNA Plasmid (h): sc-43742-SH as alternate gene silencing products.

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.

APPLICATIONS

E6-AP shRNA (h) Lentiviral Particles is recommended for the inhibition of E6-AP 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

E6-AP (E-4): sc-166689 is recommended as a control antibody for monitoring of E6-AP 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 E6-AP gene expression knockdown using RT-PCR Primer: E6-AP (h)-PR: sc-43742-PR (20 μ l, 545 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.