SANTA CRUZ BIOTECHNOLOGY, INC.

EAPP siRNA (h): sc-92116

BACKGROUND

E2F transcription factors play a major role in apoptosis and cell proliferation and are found to be frequently deregulated in cancers. Through interactions with cell cycle regulators such as cyclins, cyclin-dependent kinases and retinoblastoma protein (Rb), E2F family members also integrate cell cycle progression. EAPP (E2F-associated phosphoprotein) is a 285 amino acid highly phosphorylated nuclear protein that fine-tunes E2F activities by interacting with E2F-1, E2F-2 and E2F-3, but not E2F-4. By binding to the N-terminal domain of these E2F family members, EAPP interferes with the binding of cyclin A, Sp1 transcription factors, EBP1 and EBP2, therefore influencing E2F activity. Interestingly, EAPP is expressed during the cell cycle, but disappears during mitosis, suggesting that this step is necessary to complete the cell cycle. EAPP is ubiquitously expressed, with highest levels found in placenta, pancreas, skeletal muscle and heart.

REFERENCES

1. Ivey-Hoyle, M., et al. 1993. Cloning and characterization of E2F-2, a novel protein with the biochemical properties of transcription factor E2F. Mol. Cell. Biol. 13: 7802-7812.
2. Karlseder, J., et al. 1996. Interaction of Sp1 with the growth- and cell cycle-regulated transcription factor E2F. Mol. Cell. Biol. 16: 1659-1667.
3. Oswald, F., et al. 1996. The E2F transcription factor activates a replication-dependent human H2A gene in early S phase of the cell cycle. Mol. Cell. Biol. 16: 1889-1895.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 609486. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Araki, K., et al. 2003. Distinct recruitment of E2F family members to specific E2F-binding sites mediates activation and repression of the E2F1 promoter. Oncogene 22: 7632-7641.
6. Novy, M., et al. 2005. EAPP, a novel E2F binding protein that modulates E2F-dependent transcription. Mol. Biol. Cell 16: 2181-2190.

CHROMOSOMAL LOCATION

Genetic locus: EAPP (human) mapping to 14q13.1.

PRODUCT

EAPP 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 EAPP shRNA Plasmid (h): sc-92116-SH and EAPP shRNA (h) Lentiviral Particles: sc-92116-V as alternate gene silencing products.

For independent verification of EAPP (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-92116A, sc-92116B and sc-92116C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

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

EAPP (C-9): sc-365756 is recommended as a control antibody for monitoring of EAPP 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 EAPP gene expression knockdown using RT-PCR Primer: EAPP (h)-PR: sc-92116-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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