

PEA-15 siRNA (h): sc-37485

BACKGROUND

PEA-15 (phosphoprotein enriched in astrocytes) exists in an non-phosphorylated form (N), and two phosphorylated forms, P α and P β . PEA-15 is an endogenous substrate for PKC, which mediates the transition from P α to P β . The level of PEA-15 phosphorylation changes upon depolymerization or stabilization of tubulins, indicating that PEA-15 colocalizes with microtubules. The first 80 amino acids of PEA-15 correspond to the death effector domain (DED), which is a domain found in proteins that regulate apoptotic signaling pathways. The DED domain is necessary for PEA-15 to block Ras suppression. Although PEA-15 is predominantly expressed in the central nervous system, low levels of PEA-15 are expressed in liver and kidney, and higher levels in muscle. PEA-15 is also referred to as PED, phosphoprotein enriched in diabetes, for its elevated expression in type 2 diabetic patients.

REFERENCES

1. Araujo, H., et al. 1993. Characterization of PEA-15, a major substrate for protein kinase C in astrocytes. *J. Biol. Chem.* 268: 5911-5920.
2. Danzinger, N., et al. 1995. Cellular expression, developmental regulation, and phylogenic conservation of PEA-15, the astrocytic major phosphoprotein and protein kinase C substrate. *J. Neurochem.* 64: 1016-1025.
3. Chinnaiyan, A., et al. 1995. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. *Cell* 81: 505-512.
4. Estelles, A., et al. 1996. The major astrocytic phosphoprotein PEA-15 is encoded by two mRNAs conserved on their full length in mouse and human. *J. Biol. Chem.* 271: 14800-14806.

CHROMOSOMAL LOCATION

Genetic locus: PEA15 (human) mapping to 1q23.2.

PRODUCT

PEA-15 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PEA-15 shRNA Plasmid (h): sc-37485-SH and PEA-15 shRNA (h) Lentiviral Particles: sc-37485-V as alternate gene silencing products.

For independent verification of PEA-15 (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-37485A, sc-37485B and sc-37485C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PEA-15 siRNA (h) is recommended for the inhibition of PEA-15 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 μ M in 66 μ l. 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

PEA-15 (H-3): sc-166678 is recommended as a control antibody for monitoring of PEA-15 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 PEA-15 gene expression knockdown using RT-PCR Primer: PEA-15 (h)-PR: sc-37485-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kuramitsu, Y., et al. 2009. Proteomic differential display analysis identified upregulated astrocytic phosphoprotein PEA-15 in human malignant pleural mesothelioma cell lines. *Proteomics* 9: 5078-5089.

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.