

PAM siRNA (h): sc-106802

BACKGROUND

Peptidylglycine α -amidating monooxygenase (PAM) catalyzes the two-step formation of bioactive α -amidated neural and endocrine peptides from their glycine-extended precursors. PAM is a bifunctional protein that contains a peptidylglycine α -hydroxylating monooxygenase and a peptidyl- α -hydroxyglycine α -amidating lyase catalytic domains. Tissue-specific alternative splicing and endoproteolysis generate both soluble and integral membrane mono- and bifunctional PAM proteins. PAM is highly expressed in ovary, testis, lung, heart septum, anterior pituitary and hypothalamus, and to a lesser extent in liver, ventricle, atrium and neurointermediate lobe. The 3'-untranslated region of PAM mRNA has a novel 20-nucleotide *cis* element, which is able to interact with cellular cytosolic protease-sensitive factor. The cytosolic domain of the PAM protein contains multiple signals determining its subcellular localization. PAM interacts with three related cytosolic proteins, designated P-CIPs (PAM cytosolic interactor proteins). P-CIP2 is a protein kinase that phosphorylates PAM at serine 949. Phosphorylation of PAM in the cytosolic domain of PAM plays a critical role in the trafficking of PAM. PAM in rat sciatic nerves is proteolytically processed during the axonal transport of secretion granules.

REFERENCES

1. Husten, E.J., et al. 1993. Use of endoproteases to identify catalytic domains, linker regions, and functional interactions in soluble peptidylglycine α -amidating monooxygenase. *J. Biol. Chem.* 268: 9709-9717.
2. Yun, H.Y., et al. 1995. Phosphorylation of the cytosolic domain of peptidylglycine α -amidating monooxygenase. *J. Biol. Chem.* 270: 30075-30083.
3. Takasugi, H., et al. 1996. Distribution and processing of peptidylglycine α -mediating monooxygenase activity in rat dorsal root ganglia and sciatic nerves. *Neurochem. Int.* 29: 397-403.

CHROMOSOMAL LOCATION

Genetic locus: PAM (human) mapping to 5q21.1.

PRODUCT

PAM 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 PAM shRNA Plasmid (h): sc-106802-SH and PAM shRNA (h) Lentiviral Particles: sc-106802-V as alternate gene silencing products.

For independent verification of PAM (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-106802A, sc-106802B and sc-106802C.

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.

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

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

PAM (F-4): sc-514110 is recommended as a control antibody for monitoring of PAM 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 PAM gene expression knockdown using RT-PCR Primer: PAM (h)-PR: sc-106802-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.