

## Pam siRNA (h): sc-76038

### BACKGROUND

Ubiquitination is an important mechanism through which three classes of enzymes act in concert to target short-lived or abnormal proteins for destruction. The three classes of enzymes involved in ubiquitination are the ubiquitin-activating enzymes (E1s), the ubiquitin-conjugating enzymes (E2s) and the ubiquitin-protein ligases (E3s). Pam, also known as MYCBP2 (Myc-binding protein 2), is a 4,640 amino acid nuclear protein that contains one B box-type zinc finger, one DOC domain, one filamin repeat, five RCC1 repeats and one RING-type zinc finger. Expressed ubiquitously with highest expression in thymus and brain, Pam is thought to function as an E3 ubiquitin-protein ligase that may be involved in the degradation of a variety of target proteins. Additionally, Pam may also be involved in regulating c-Myc expression, possibly influencing synaptogenesis. Two isoforms of Pam exist due to alternative splicing events.

### REFERENCES

1. Borden, K.L. and Freemont, P.S. 1996. The RING finger domain: a recent example of a sequence-structure family. *Curr. Opin. Struct. Biol.* 6: 395-401.
2. Guo, Q., Xie, J., Dang, C.V., Liu, E.T. and Bishop, J.M. 1998. Identification of a large Myc-binding protein that contains RCC1-like repeats. *Proc. Natl. Acad. Sci. USA* 95: 9172-9177.
3. Lorick, K.L., Jensen, J.P., Fang, S., Ong, A.M., Hatakeyama, S. and Weissman, A.M. 1999. RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc. Natl. Acad. Sci. USA* 96: 11364-11369.
4. Pierre, S.C., Häusler, J., Birod, K., Geisslinger, G. and Scholich, K. 2004. PAM mediates sustained inhibition of cAMP signaling by sphingosine-1-phosphate. *EMBO J.* 23: 3031-3040.
5. Murthy, V., Han, S., Beauchamp, R.L., Smith, N., Haddad, L.A., Ito, N. and Ramesh, V. 2004. Pam and its ortholog highwire interact with and may negatively regulate the TSC1.TSC2 complex. *J. Biol. Chem.* 279: 1351-1358.
6. Gao, X. and Patel, T.B. 2005. Histidine residues 912 and 913 in protein associated with Myc are necessary for the inhibition of adenyl cyclase activity. *Mol. Pharmacol.* 67: 42-49.

### CHROMOSOMAL LOCATION

Genetic locus: MYCBP2 (human) mapping to 13q22.3.

### 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Pam shRNA Plasmid (h): sc-76038-SH and Pam shRNA (h) Lentiviral Particles: sc-76038-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-76038A, sc-76038B and sc-76038C.

### 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ 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  $\mu$ M in 66  $\mu$ 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.

### RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Pam gene expression knockdown using RT-PCR Primer: Pam (h)-PR: sc-76038-PR (20  $\mu$ l, 485 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

### RESEARCH USE

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

### PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.