



# AWP1 siRNA (h): sc-62004

## BACKGROUND

AWP1 (associated with PKN protein), also known as zinc finger A20 domain-containing protein 3 (ZA20D3) or AN1-type zinc finger protein 6 (ZFAND6 or ZFAND5B), is a ubiquitously expressed protein with highest expression levels in liver, kidney, placenta, heart and skeletal muscle. AWP1 contains two conserved domains (one AN1-type zinc finger and one A20-type zinc finger), two PEST sequences, seven casein kinase II phosphorylation sites, two N-myristoylation sites and four N-glycosylation sites. The AN1-type zinc finger and four cysteine residues near the N-terminus are conserved between AWP1, OSISAP1 and ZNF216. Human AWP1 shares 55% homology with ZNF216 and mouse AWP1. It is a potent inhibitor of NF $\kappa$ B and it interacts with PKN, possibly participating in the regulation of PKN signal transduction pathways. Two AWP1 isoforms exist, due to alternative splicing. Isoform 1 is the mature full length protein and isoform 2 lacks amino acids 52-63.

## REFERENCES

- Williams, K.J., et al. 1996. Development of a PCR-based allele-specific assay from an RFLP probe linked to resistance to cereal cyst nematode in wheat. *Genome* 39: 798-801.
- Duan, W., et al. 2000. Cloning and characterization of AWP1, a novel protein that associates with serine/threonine kinase PRK1 *in vivo*. *Gene* 256: 113-121.
- Mukhopadhyay, A., et al. 2004. Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. *Proc. Natl. Acad. Sci. USA* 101: 6309-6314.
- Cao, Y.K., et al. 2005. Construction of GFP-AWP1 fusion gene vector and its expression in 293 cells. *Di Yi Jun Yi Da Xue Xue Bao* 25: 174-176, 180.

## CHROMOSOMAL LOCATION

Genetic locus: ZFAND6 (human) mapping to 15q25.1.

## PRODUCT

AWP1 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 AWP1 shRNA Plasmid (h): sc-62004-SH and AWP1 shRNA (h) Lentiviral Particles: sc-62004-V as alternate gene silencing products.

For independent verification of AWP1 (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-62004A, sc-62004B and sc-62004C.

## 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

AWP1 siRNA (h) is recommended for the inhibition of AWP1 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.

## GENE EXPRESSION MONITORING

AWP1 (B-5): sc-398513 is recommended as a control antibody for monitoring of AWP1 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 AWP1 gene expression knockdown using RT-PCR Primer: AWP1 (h)-PR: sc-62004-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

- Fenner, B.J., et al. 2009. Identification of polyubiquitin binding proteins involved in NF $\kappa$ B signaling using protein arrays. *Biochim. Biophys. Acta* 1794: 1010-1016.

## 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.