

# PAR6A siRNA (h): sc-40809

## BACKGROUND

Cellular asymmetry is critical for the development of multicellular organisms. PARD (partitioning-defective) proteins play important roles in asymmetric cell division and polarized growth, whereas Cdc42 and Rac mediate establishment of cell growth and polarity and contribute to oncogenic transformation by Ras. The human PARD6, a 345 amino acid polypeptide, has a PDZ domain and a CRIB-like (Cdc42/Rac interactive binding) motif. PARD6 interacts with GTP-bound Rac and Cdc42 via this motif and with the atypical PKC isoforms PKC $\lambda$  and PKC $\zeta$  via N-terminal head to head association. These interactions allow formation of a ternary complex *in vitro* and *in vivo*, which is implicated in the formation of normal tight junctions at epithelial cell-cell contacts and is also involved in the polarization of mother cells before asymmetric cell division in *C. elegans*. PARD6 acts through PARD3 by localizing or maintaining the PARD3 protein at the cell periphery. PARD6A, also designated PAR-6 $\alpha$ , PAR6C, TAX40 and TIP-40, is expressed in pancreas, skeletal muscle, brain and heart, and is weakly expressed in kidney and placenta. PAR6B is expressed in pancreas and in both adult and fetal kidney, and is weakly expressed in placenta and lung.

## REFERENCES

1. Watts, J.L., et al. 1996. PAR-6, a gene involved in the establishment of asymmetry in early *C. elegans* embryos, mediates the asymmetric localization of PAR-3. *Development* 122: 3133-3140.
2. Kim, S.K. 2000. Cell polarity: new PARTners for Cdc42 and Rac. *Nat. Cell Biol.* 2: 143-145.
3. Joberty, G., et al. 2000. The cell-polarity protein PAR-6 links PAR-3 and atypical protein kinase C to Cdc42. *Nat. Cell Biol.* 2: 531-539.
4. Lin D., et al. 2000 A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signaling and cell polarity. *Nat. Cell Biol.* 2: 540-547.
5. Qiu, R.G., et al. 2000. A human homolog of the *C. elegans* polarity determinant PAR-6 links Rac and Cdc42 to PKC $\zeta$  signaling and cell transformation. *Curr. Biol.* 10: 697-707.
6. Brazil, D.P., et al. 2000 Cell polarity: scaffold proteins PAR excellence. *Curr. Biol.* 10: R592-R594.

## CHROMOSOMAL LOCATION

Genetic locus: PARD6A (human) mapping to 16q22.1.

## PRODUCT

PAR6A 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 PAR6A shRNA Plasmid (h): sc-40809-SH and PAR6A shRNA (h) Lentiviral Particles: sc-40809-V as alternate gene silencing products.

For independent verification of PAR6A (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-40809A, sc-40809B and sc-40809C.

## 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

PAR6A siRNA (h) is recommended for the inhibition of PAR6A 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

PAR6A (C-3): sc-365323 is recommended as a control antibody for monitoring of PAR6A 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<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> 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<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PAR6A gene expression knockdown using RT-PCR Primer: PAR6A (h)-PR: sc-40809-PR (20  $\mu$ l, 512 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.