

# PERK siRNA (r): sc-60074

## BACKGROUND

An interferon-inducible, RNA-dependent protein serine/threonine kinase (PKR) has been described. PKR in earlier literature is variously known as DAI, dsJ, PI kinase, p65, p67 or TIK for the mouse kinase; and p68 or p69 for the human kinase. The PKR kinase substrate is the  $\alpha$  subunit of protein synthesis initiation factor eIF2. Phosphorylation of eIF2 $\alpha$  on Serine 51 results in inhibition of translation. The serine/threonine kinase catalytic domains map to the carboxy terminal half of the protein while the RNA-binding domains are located in the amino terminal region. PERK is a type I transmembrane protein located in the endoplasmic reticulum (ER) that contains a kinase domain similar to the kinase domain of PKR. PERK is activated in response to ER stress and phosphorylates eIF2 $\alpha$ , thus inhibiting the translation of mRNA.

## REFERENCES

- Hershey, J.W.B., et al. 1989. Protein phosphorylation controls translation rates. *J. Biol. Chem.* 264: 20823-20826.
- Meurs, E., et al. 1990. Molecular cloning and characterization of the human double-stranded RNA-activated protein kinase induced by interferon. *Cell* 62: 379-390.
- Thomis, D.C., et al. 1992. Mechanism of interferon action: cDNA structure expression and regulation of the interferon-induced, RNA-dependent PI/eIF2 $\alpha$  protein kinase from human cells. *Virology* 188: 33-46.
- McCormack, S.J., et al. 1992. Mechanism of interferon action: identification of a RNA binding domain within the N-terminal region of the human RNA-dependent PI/eIF2 $\alpha$  protein kinase. *Virology* 188: 47-56.

## CHROMOSOMAL LOCATION

Genetic locus: Eif2ak3 (rat) mapping to 4q31.

## PRODUCT

PERK siRNA (r) 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 PERK shRNA Plasmid (r): sc-60074-SH and PERK shRNA (r) Lentiviral Particles: sc-60074-V as alternate gene silencing products.

For independent verification of PERK (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-60074A, sc-60074B and sc-60074C.

## 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

PERK siRNA (r) is recommended for the inhibition of PERK expression in rat 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

PERK (B-5): sc-377400 is recommended as a control antibody for monitoring of PERK 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 PERK gene expression knockdown using RT-PCR Primer: PERK (r)-PR: sc-60074-PR (20  $\mu$ l, 492 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

- Hao, Y., et al. 2019. Unfolded protein response is involved in geniposide-regulating glucose-stimulated insulin secretion in INS-1 cells. *Cell Biochem. Funct.* 37: 368-376.

## 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.