SANTA CRUZ BIOTECHNOLOGY, INC.

PACT siRNA (m2): sc-63342

BACKGROUND

Interferon-inducible double stranded RNA-dependent protein kinase activator, also designated PKR-associated protein X (RAX) or PACT, acts as a protein activator of PKR. Following stress such as serum starvation or peroxide or arsenite treatment, PACT associates with and activates PKR, resulting in eIF2α activation (phosphorylation), consequent translation inhibition and apoptosis. PACT can directly interact with double stranded RNA (dsRNA), however, eIF2α activation occurs only in the absence of dsRNA. The presence of certain growth factors may suppress the pro-apoptotic function of PACT. In both human and mouse cells, PACT is phosphorylated on Serine 18, and the phosphorylated form activates PKR following stress. PACT may exist as a heterodimer with eIF2α, interacting through its DRBM domain.

REFERENCES

1. Patel, R.C., et al. 1998. PACT, a protein activator of the interferon-induced protein kinase, PKR. EMBO J. 17: 4379-4390.
2. Ito, T., et al. 1999. RAX, a cellular activator for double-stranded RNA-dependent protein kinase during stress signaling. J. Biol. Chem. 274: 15427-15432.
3. Huang, X., et al. 2002. The C-terminal, third conserved motif of the protein activator PACT plays an essential role in the activation of double stranded RNA-dependent protein kinase (PKR). Biochem. J. 366: 175-186.
4. Peters, G.A., et al. 2002. Inhibition of PACT-mediated activation of PKR by the herpes simplex virus type 1 Us11 protein. J. Virol. 76: 11054-11064.
5. Yang, M., et al. 2003. A novel role for RAX, the cellular activator of PKR, in synergistically stimulating SV40 large T antigen-dependent gene expression. J. Biol. Chem. 278: 38325-38332.
6. Bennett, R.L., et al. 2004. Serine 18 phosphorylation of RAX, the PKR activator, is required for PKR activation and consequent translation inhibition. J. Biol. Chem. 279: 42687-42693.

CHROMOSOMAL LOCATION

Genetic locus: Prkra (mouse) mapping to 2 C3.

PRODUCT

PACT siRNA (m2) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PACT shRNA Plasmid (m2): sc-63342-SH and PACT shRNA (m2) Lentiviral Particles: sc-63342-V as alternate gene silencing products.

For independent verification of PACT (m2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-63342A, sc-63342B and sc-63342C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PACT siRNA (m2) is recommended for the inhibition of PACT expression in mouse 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 µM in 66 µl. 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

PACT (D-4): sc-377103 is recommended as a control antibody for monitoring of 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 gene expression knockdown using RT-PCR Primer: PACT (m2)-PR: sc-63342-PR (20 µl, 554 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.