

# FKHR siRNA (r): sc-270624

## BACKGROUND

FKHR (for forkhead in rhabdomyosarcoma) and FKHL1 are members of the forkhead family of transcription factors. Transcriptional activation of FKHR proteins is regulated by the serine/threonine kinase Akt1, which phosphorylates FKHL1 and results in FKHL1 associating with 14-3-3 proteins and being retained in the cytoplasm. Induction of apoptosis or withdrawal of growth factors stimulates dephosphorylation and nuclear translocation of FKHR proteins, leading to FKHR-induced gene-specific transcriptional activation. FKHR, also designated forkhead box protein O1A (FOXO1), is a ubiquitously expressed protein that shuttles between the cytoplasm and nucleus. Genetic mutations in FKHR genes, including the t(2;13) and t(1;3) translocations, are commonly found in alveolar rhabdomyosarcomas. These translocations result in the fusion of the amino terminus of Pax-3 or Pax-7, including the paired box and homeodomain DNA-binding domains, with the carboxy-terminus of FKHR, which contains a transcriptional activation domain. The Pax-3/FKHR fusion protein appears to function as an oncogenic transcription factor that enhances the activation of normal Pax-3 target genes.

## REFERENCES

- Galili, N., et al. 1993. Fusion of a fork head domain gene to Pax-3 in the solid tumour alveolar rhabdomyosarcoma. *Nat. Genet.* 5: 230-235.
- Davis, R.J., et al. 1995. Structural characterization of the FKHR gene and its rearrangement in alveolar rhabdomyosarcoma. *Hum. Mol. Genet.* 4: 2355-2362.
- Anderson, M.J., et al. 1998. Cloning and characterization of three human forkhead genes that comprise an FKHR-like gene subfamily. *Genomics* 47: 187-199.
- Tang, E.D., et al. 1999. Negative regulation of the forkhead transcription factor FKHR by Akt. *J. Biol. Chem.* 274: 16741-16746.
- Rena, G., et al. 1999. Phosphorylation of the transcription factor forkhead family member FKHR by protein kinase B. *J. Biol. Chem.* 274: 17179-17183.
- Brunet, A., et al. 1999. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 96: 857-868.

## CHROMOSOMAL LOCATION

Genetic locus: Foxo1 (rat) mapping to 2q26.

## PRODUCT

FKHR 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 FKHR shRNA Plasmid (r): sc-270624-SH and FKHR shRNA (r) Lentiviral Particles: sc-270624-V as alternate gene silencing products.

For independent verification of FKHR (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-270624A, sc-270624B and sc-270624C.

## 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

FKHR siRNA (r) is recommended for the inhibition of FKHR 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

FKHR (C-9): sc-374427 is recommended as a control antibody for monitoring of FKHR 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 FKHR gene expression knockdown using RT-PCR Primer: FKHR (r)-PR: sc-270624-PR (20  $\mu$ l). 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.