SANTA CRUZ BIOTECHNOLOGY, INC.

p-FKHR (Ser 319): sc-19807

BACKGROUND

The transcription factor forkhead in rhabdomyosarcoma (FKHR), which is inhibited by insulin and IGF-1, enhances transcription. FKHR has been implicated in alveolar rhabdomyosarcoma, a soft tissue tumor wherein a chromosomal translocation [t(2;12)(q35;q14)] occurs between the FKHR and PAX3 genes, resulting in a novel chimeric protein with abnormal levels of expression. FKHR becomes phosphorylated at Ser 319, Ser 256 and Thr 24 by protein kinase B (PKB) in a phosphoinsoditide 3-(PI3) kinase/Akt dependent pathway, resulting in the inactivation and subsequent nuclear exit of FKHR. In addition, FKHR becomes phosphorylated at Ser 329, also resulting in decreased FKHR activity and diminished nuclear FKHR concentration. However, phosphorylation of FKHR at Ser 329 is not mediated by a PI3-kinase-dependent pathway, but by an alternate mechanism. Dual-specificity tyrosine-phosphorylated and regulated kinase 1A (DYRK1A), which co-localizes to the same region of the nucleus as FKHR, specifically phosphorylates FKHR at Ser 329 in rabbit skeletal muscle.

REFERENCES

1. Pappo, A.S., et al. 1995. Biology and therapy of pediatric rhabdomyosarcoma. J. Clin. Oncol. 13: 2123-2139.
2. Rena, G., et al. 1999. Phosphorylation of the transcription factor forkhead family member FKHR by protein kinase B. J. Biol. Chem. 274: 17179-17183.
3. Nakae, J., et al. 2000. Differential regulation of gene expression by Insulin and IGF-1 receptors correlates with phosphorylation of a single amino acid residue in the forkhead transcription factor FKHR. EMBO J. 19: 989-996.
4. Nakae, J., et al. 2001. Insulin regulation of gene expression through the forkhead transcription factor Foxo1 (FKHR) requires kinases distinct from Akt. Biochemistry 40: 11768-11776.
5. Rena, G., et al. 2001. Roles of the forkhead in rhabdomyosarcoma (FKHR) phosphorylation sites in regulating 14-3-3 binding, transactivation and nuclear targetting. Biochem. J. 354: 605-612.
6. Woods, Y.L., et al. 2001. The kinase Dyrk1A phosphorylates the transcription factor FKHR at Ser 329 *in vitro,* a novel *in vivo* phosphorylation site. Biochem. J. 355: 597-607.

CHROMOSOMAL LOCATION

Genetic locus: FOXO1 (human) mapping to 13q14.11; Foxo1 (mouse) mapping to 3 C.

SOURCE

p-FKHR (Ser 319) is available as either goat (sc-19807) or rabbit (sc-19807-R) polyclonal antibody raised against a short amino acid sequence containing Ser 319 phosphorylated FKHR of human origin.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

PRODUCT

Each vial contains 200 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-19807 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

APPLICATIONS

p-FKHR (Ser 319) is recommended for detection of Ser 319 phosphorylated FKHR of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

p-FKHR (Ser 319) is also recommended for detection of correspondingly phosphorylated FKHR in additional species, including equine, canine, bovine, porcine and avian.

Suitable for use as control antibody for FKHR siRNA (h): sc-35382, FKHR siRNA (m): sc-35383, FKHR shRNA Plasmid (h): sc-35382-SH, FKHR shRNA Plasmid (m): sc-35383-SH, FKHR shRNA (h) Lentiviral Particles: sc-35382-V and FKHR shRNA (m) Lentiviral Particles: sc-35383-V.

Molecular Weight of p-FKHR: 80 kDa.

Positive Controls: NIH/3T3 + serum cell lysate: sc-2248.

RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: for goat primary antibody (sc-19807): use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), for rabbit primary antibody (sc-19807-R): use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Western Blotting Luminol Reagent: sc-2048 and Lambda Phosphatase: sc-200312A. 2) Immunofluorescence: for goat primary antibody (sc-19807): use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941, for rabbit primary antibody (sc-19807-R): use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

SELECT PRODUCT CITATIONS

1. Gan, L., et al. 2005. Nuclear/cytoplasmic shuttling of the transcription factor FoxO1 is regulated by neurotrophic factors. J. Neurochem. 93: 1209-1219.

RESEARCH USE

For research use only, not for use in diagnostic procedures.