

p-AFX1 (Ser 197): sc-101628

BACKGROUND

FKHR (for forkhead in rhabdomyosarcoma), FKHL1, and AFX1 are members of a subfamily of the forkhead family of transcription factors. AFX1, also known as FoxO4, is expressed in a wide variety of tissues and, like other FKHR proteins, AFX1 contains a single forkhead domain and serine-proline-rich region, which mediate DNA binding. AFX1-mediated transcriptional activation is regulated by the serine/threonine kinase Akt1, which phosphorylates AFX1 (in humans, this phosphorylation can occur at several sites, including Ser 197) and in turn, sequesters AFX1 in the cytosol, thereby blocking nuclear localization and DNA binding. Genetic mutations in FKHR genes, including the t(2;13) and t(1;3) translocations, are commonly found in alveolar rhabdomyosarcomas. Additionally, the t(x;11) translocation of the AFX1 gene, which involves the fusion of a serine-proline-rich sequence of AFX1 to the carboxy-terminus of a truncated MLL, results in acute lymphocytic leukemia.

REFERENCES

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2. Parry, P., et al. 1994. Cloning and characterization of the t(X;11) breakpoint from a leukemic cell line identify a new member of the forkhead gene family. *Genes Chromosomes Cancer* 11: 79-84.
3. Davis, R.J., et al. 1995. Structural characterization of the FKHR gene and its rearrangement in alveolar rhabdomyosarcoma. *Hum. Mol. Genet.* 4: 2355-2362.
4. Peters, U., et al. 1997. AFX1 and p54nrb: fine mapping, genomic structure, and exclusion as candidate genes of X-linked dystonia parkinsonism. *Hum. Genet.* 100: 569-572.
5. 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.
6. Kops, G.J., et al. 1999. Direct control of the forkhead transcription factor AFX by protein kinase B. *Nature* 398: 630-634.
7. 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: FOXO4 (human) mapping to Xq13.1; Foxo4 (mouse) mapping to X C3.

SOURCE

p-AFX1 (Ser 197) is a rabbit polyclonal antibody raised against a short amino acid sequence containing phosphorylated Ser 197 of AFX1 of human origin.

PRODUCT

Each vial contains 100 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

APPLICATIONS

p-AFX1 (Ser 197) is recommended for detection of Ser 197 phosphorylated AFX1 of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1–2 µg per 100–500 µg of total protein (1 ml of cell lysate)], immunofluorescence and immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500).

Suitable for use as control antibody for AFX1 siRNA (h): sc-29650 and AFX1 siRNA (m): sc-29651.

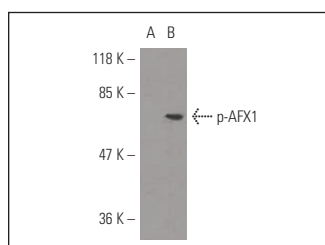
Molecular Weight of p-AFX1: 60 kDa.

Positive Controls: serum-treated 293 whole cell lysate or human breast carcinoma tissue.

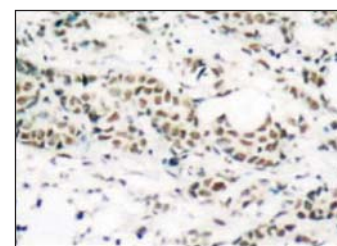
RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: 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) and Western Blotting Luminol Reagent: sc-2048. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: 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. 4) Immunohistochemistry: use ImmunoCruz™: sc-2051 or ABC: sc-2018 rabbit IgG Staining Systems.

DATA



p-AFX1 (Ser 197): sc-101628. Western blot analysis of phosphorylated AFX1 expression in untreated (A) and serum-treated (B) 293 whole cell lysates.



p-AFX1 (Ser 197): sc-101628. Immunoperoxidase staining of formalin-fixed, paraffin-embedded human breast carcinoma tissue showing nuclear staining.

STORAGE

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

RESEARCH USE

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

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.