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ProductInformation

Anti-AFX (FOXO4)
Developed in Rabbit
Affinity Isolated Antibody

Product Number **A 8975**

Product Description

Anti-AFX (FOXO4) is developed in rabbit using a synthetic peptide corresponding to the C-terminus of human AFX (amino acids 451-466 with N-terminally added lysine) conjugated to KLH as immunogen. Anti-AFX is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-AFX (FOXO4) recognizes human AFX by immunoblotting (approx. 50 kDa) and indirect immunofluorescence. Staining of AFX is specifically inhibited by the AFX immunizing peptide.

The superfamily of Forkhead transcription factors (FOX) consists of more than 100 members, with orthologues expressed in a variety of species ranging from yeast to man.[1,2] They are characterized by a common Forkhead (or Winged Helix) domain, a variant of the helix-turn-helix motif.[2,3] Forkhead family members have been shown to play key regulatory roles in embryogenic development, differentiation, apoptosis, and tumorigenesis.[1-5] Three Forkhead family members, termed FKHR (FOXO1a), FKHRL1 (FOXO3a), and AFX (FOXO4) were first identified at chromosomal breakpoints in human tumors, and consequently linked to tumorigenesis.[5-8]

The key to understanding the function of these proteins was the finding that they represent the mammalian counterparts of DAF16, which transduces insulin-like and longevity signals in the nematode *C. elegans*.[9,10] Similar to DAF-16, FKHR, and FKHRL1, AFX have three putative sites for PKB/AKT phosphorylation which plays a central role in the regulation of their activity.[11-13] AFX contains three putative PKB phosphorylation sites at Thr^{28}, Ser^{193}, and Ser^{258}. Insulin induces the phosphorylation of AFX by way of both PI3K/PKB and Ras/Ral signaling pathways.[12] Phosphorylation of AFX by PKB, after induction by growth factors, alters its steady-state distribution. Initially, PKB is activated and translocated to the nucleus; AFX is then phosphorylated, ultimately leading to its export to the cytoplasm.[14] Altogether, phosphorylation of AFX by survival factors results in inhibition of its transcriptional activity. Withdrawal of survival factors results in AFX dephosphorylation, nuclear localization, and target gene activation.[11-13]
AFX also plays a central role in the regulation of cell proliferation. It integrates signals from the PI3K/PKB and Ras/Rals signaling pathways to regulate transcription of the cell cycle inhibitor $p27^{kip1}$. AFX activation results in increased expression of $p27^{kip1}$, resulting in cell cycle arrest. Overexpression of AFX blocks cells cycle progression at G1 by a $p27^{kip1}$ dependent mechanism.[14,15] Inactivation of Forkhead proteins may thus comprise an important step in oncogenic transformation by both, inhibiting apoptosis and promoting progression through the cell cycle.[15,16]
An isoform of AFX, named AFXζ, has recently been isolated and is regulated through both PI3K/PKB as well as through the AMP-activated protein kinase cascade.[17]

Antibodies reacting specifically with AFX (FOXO4) may be useful for studying the expression and function of the protein, as well as for correlating their expression pattern with physiological functions or pathological conditions.

Reagent

Anti-AFX (FOXO4) is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin (BSA) and 15 mM sodium azide.

Antibody concentration: minimum 0.8 mg/ml

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability
For continuous use, store at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at –20 °C. Repeated freezing and thawing is not recommended. Storage in frost-free freezers is also not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile
A minimum working dilution of 1:2,000 is determined by immunoblotting using human AFX expressed in transfected COS-7 cell extracts.

A minimum working dilution of 1:1,000 is determined by indirect immunofluorescence using paraformaldehyde-fixed AFX transfected COS-7 cells.

Note: In order to obtain the best results using different techniques and preparations, we recommend determining the optimal working dilutions by titration.

References

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