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Product Information

Anti-AFX (FOXO4) antibody
Mouse monoclonal, clone AF3.10
purified from hybridoma cell culture

Product Number **A5854**

Product Description

Anti-AFX (FOXO4) antibody, Mouse monoclonal (mouse IgG2b isotype) is derived from the hybridoma AF3.10 produced by the fusion of mouse myeloma cells (NS1 cells) and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to a sequence in the C-terminus of human AFX with N-terminally added cysteine, conjugated to KLH. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Product Number ISO2.

Anti-AFX (FOXO4) antibody, Mouse monoclonal recognizes human AFX. The antibody may be used in ELISA, immunoblotting (~50 kDa), and immunocytochemistry.

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] These molecules 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, the Forkhead transcription factors FKHR, FKHRL1, and AFX have three putative sites for PKB/AKT phosphorylation, which play 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 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] In addition, AFX plays a central role in the regulation of cell proliferation. It integrates signals from 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 G_1 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]

Interestingly, an isoform of AFX, named AFXζ, has been isolated. It is regulated through both PI3K/PKB as well as 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 its expression pattern with physiological functions or pathological conditions.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: ~2 mg/mL.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet 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. Repeated freezing and thawing, or storage in frost-free freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile
Immunoblotting: a working antibody concentration of 0.5-1 μg/mL is recommended using COS-7 cell extracts expressing human AFX.

Note: In order to obtain best results in various techniques and preparations, it is recommended to determine optimal working dilutions by titration.

References

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