

Product Information

Anti-FKHRL1 (FOXO3a)

Developed in Rabbit
Affinity Isolated Antibody

Product Number **F 2178**

Product Description

Anti-FKHRL1 (FOXO3a) is developed in rabbit using a synthetic peptide corresponding to the N-terminus of human FKHRL1 (amino acids 653-668 with N-terminally added cystein) conjugated to maleimide-activated KLH as immunogen. This sequence is conserved in human and mouse FKHRL1. Anti-FKHRL1 is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-FKHRL1 recognizes human and mouse FKHRL1 (70-75 kDa). Applications include immunoblotting and indirect immunofluorescence. In some preparations, there is an additional band at 35 kDa. Staining of FKHRL1 is specifically inhibited with the FKHRL1 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 embryonic 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 It is now well established that these proteins are targets of the PI3K/PKB pathway.

PI3K/PKB (Phosphatidylinositol 3-kinase/Protein Kinase B or Akt) plays a role in oncogenic transformation. PKB/Akt substrates include components of the cell death machinery, such as BAD and caspase 9.^{1,9,10} Stimulation of this cascade by Nerve Growth Factor or IGF-1 leads to phosphorylation of these proteins and suppression of their proapoptotic function, partially explaining the survival effect of PKB.^{11,12} The identification of the transcription factor DAF16 as a PKB target in the nematode *C. elegans* was critical in the understanding of its link with FKHR, FKHRL1, and AFX.¹³ DAF16 belongs to the Forkhead family and transduces insulin-like signals.^{13,14} FKHR, FKHRL1, and AFX (FOXO4)^{1,5} are similar in sequence

to DAF16 and represent the mammalian counterparts.¹⁴ Similarly, these proteins are PKB/AKT targets.¹⁵⁻¹⁷

Growth factors regulate the activity of FKHRL1, FKHR, and AFX via the PKB/PI3K pathway, by direct phosphorylation of the transcription factors.^{3,15-17} These transcription factors are inhibited by phosphorylation by PKB, the most likely mechanism being regulation of nuclear localization.¹⁴⁻¹⁶ In FKHRL1 (FOXO3a), there are three PKB phosphorylation consensus sites, Thr³², Ser²⁵³, and Ser³¹⁵. Thr³² and Ser²⁵³ are phosphorylated by PKB after induction by survival factors such as IGF-1. This results in FKHRL1 retention in the cytoplasm, and/or nuclear exclusion, and consequent inhibition of FKHRL1-dependent transcription.¹⁷

Survival factor withdrawal induces FKHRL1 dephosphorylation and translocation to the nucleus. Within the nucleus, the dephosphorylated FKHRL1 induces target genes such as Fas ligand, and triggers apoptosis.¹⁷ Growth factors suppress the transcription of death genes by triggering PKB/AKT dependent phosphorylation and inactivation of FKHRL1, and thereby promote cell survival. A similar mechanism is proposed for FKHR and AFX.^{16,17} It has been shown that FKHRL1/FOXO3a modulates the expression of several genes that regulate DNA repair in response to stress at the G2-M checkpoint, oxidative stress resistance, and aging.¹⁸⁻²⁰ Other genes that are regulated by FKHRL1 include mitotic genes such as cyclin B and polo-like kinase (plk).²¹

Antibodies reacting specifically with FKHRL1 (FOXO3) may be useful in studying the expression and function of the protein, as well as for correlating their expression pattern with physiological functions or pathological conditions.

Reagent

Anti-FKHRL1 (FOXO3a) 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 1:2,500 is determined by immunoblotting using human FKHRL1 in transfected 293T cell extracts.

A minimum working dilution of 1:200 is determined by immunofluorescence staining of paraformaldehyde-fixed FKHRL1 transfected 293T 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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