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ProductInformation

Monoclonal Anti-FKHRL1 (FOXO3a)
Clone FR1
Purified Mouse Immunoglobulin

Product Number **F1304**

Product Description

Monoclonal Anti-FKHRL1 (FOXO3a) (mouse IgG1 isotype) is derived from the FR1 hybridoma produced by the fusion of mouse myeloma cells (NS1 cells) and splenocytes from BALB/c mice immunized with a synthetic peptide corresponding to amino acids 653-668 of human FKHRL1, conjugated to KLH. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal anti-FKHRL1 (FOXO3a) recognizes human FKHRL1 (approx. 75 kDa). The antibody epitope resides within amino acids 653-668 of human FKHRL. The antibody may be used in ELISA, immunoblotting, 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] 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 embryogenesis, differentiation, apoptosis and tumorigenesis.[1-4] 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.[4-7] It is now well established that these proteins are targets of the PI3K/PKB pathway. The PI3K/PKB (Phosphatydil inositol 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, 8, 9] 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.[10, 11] 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.[12] DAF16 belongs to the Forkhead family and transduces insulin-like signals.[12, 13] FKHR, FKHRL1 and AFX (FOXO4)[1, 4] are similar in sequence to DAF16 and represent the mammalian counterparts.[13] Similarly, these proteins are PKB/AKT targets.[14, 15]

Growth factors regulate the activity of FKHRL1, FKHR, and AFX via the PKB/PI3K pathway, by direct phosphorylation of the transcription factors.[3, 14, 15] These transcription factors are inhibited by phosphorylation by PKB, the most likely mechanism being regulation of nuclear localization.[13,14] In FKHRL1 (FOX3a), there are three PKB phosphorylation consensus sites, Thr^{32} and Ser^{253} and Ser^{315}. Thr^{32} and Ser^{253} 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.[15] 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.[15] Growth factors, by triggering the PKB/AKT dependent phosphorylation and inactivation of FKHRL1 function, suppress the transcription of death genes, and thereby promote cell survival. A similar mechanism is proposed for FKHR and AFX.[14,15] Lately, 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, the regulation of cellular oxidative stress resistance and aging.[16] Other genes that are regulated by FKHRL1 include mitotic genes such as cyclin B and polo-like kinase (plk).[17]

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

Monoclonal Anti-FKHRL1 (FOXO3a) is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: Approx. 2 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. 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 dilution samples should be discarded if not used within 12 hours.

Product Profile
By immunoblotting, a working antibody concentration of 0.25-0.5 µg/ml is recommended using cell extracts of COS7 cells expressing human FKHRL1.

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

References

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