Background: The Forkhead family of transcription factors is involved in tumorigenesis of rhabdomyosarcoma and acute leukemias (1-3). Within the family, three members (FoxO1, FoxO4, and FoxO3a) have sequence similarity to the nematode orthologue DAF-16, which mediates signaling via a pathway involving IGFR1, PI3K, and Akt (4-6). Active forkhead members act as tumor suppressors by promoting cell cycle arrest and apoptosis. Increased expression of any FoxO member results in the activation of the cell cycle inhibitor p27 Kip1. Forkhead transcription factors also play a part in TGF-β-mediated upregulation of p21 Cip1, a process negatively regulated through PI3K (7). Increased proliferation results when forkhead transcription factors are inactivated through phosphorylation by Akt at Thr24, Ser256, and Ser319, which results in nuclear export and inhibition of transcription factor activity (8). Forkhead transcription factors can also be inhibited by the deacetylase sirtuin (Sirt1) (9).

Specificity/Sensitivity: Phospho-FoxO1 (Thr24)/FoxO3a (Thr32)/FoxO4 (Thr28) (4G6) Rabbit mAb detects endogenous levels of FoxO1 when phosphorylated at Thr24, of FoxO3a when phosphorylated at Thr32 or FoxO4 when phosphorylated at Thr28.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr28 of human FoxO4.

Recommended Antibody Dilutions: Western blotting 1:1000

Western blot analysis of extracts from Jurkat cells treated with either Calyculin A (#9902) or LY294002 (#9901) using Phospho-FoxO1 (Thr24)/FoxO3a (Thr32)/FoxO4 (Thr28) (4G6) Rabbit mAb. The phospho-specificity of the antibody was verified by treating the membrane in the absence (-) or presence (+) of calf intestinal phosphatase (CIP) after western transfer.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

Background References:
(9) Yang, Y. et al. (2005) EMBO J 24, 1021-32.

**Species cross-reactivity is determined by western blot.

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