

# Phospho-FoxO3a (Ser294) Antibody



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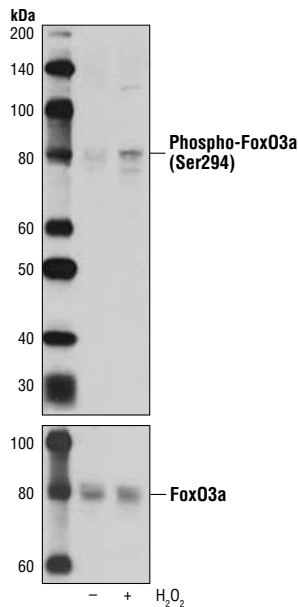
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W, IP Endogenous	H, M, R, Mk	82-97 kDa	Rabbit**

**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 IGF1R, 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 p27Kip1. Forkhead transcription factors also play a part in TGF- $\beta$ -mediated upregulation of p21CIP1, 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).

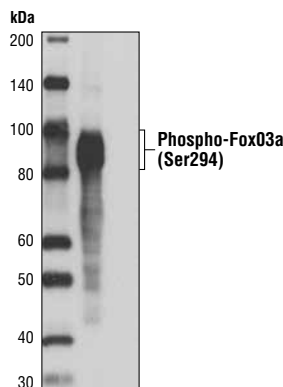
Erk phosphorylates FoxO3a at Ser294, Ser344 and Ser425, resulting in degradation of FoxO3a through the MDM2-mediated ubiquitin-proteasome pathway. Thus, Erk promotes proliferation and tumor progression by inhibiting FoxO3a (10).

**Specificity/Sensitivity:** Phospho-FoxO3a (Ser294) Antibody detects exogenous and endogenous levels of FoxO3a protein only when phosphorylated at serine 294.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic peptide surrounding Ser294 of human FoxO3a. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from C2C12 cells, untreated or H<sub>2</sub>O<sub>2</sub> treated, using Phospho-FoxO3a (Ser294) Antibody (upper) or FoxO3a (75D8) Rabbit mAb #2497 (lower).



Western blot analysis of extracts from 293T cells, transfected with tagged FoxO3a, using Phospho-FoxO3a (Ser294) Antibody.

**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

Entrez-Gene ID #2309  
Swiss-Prot Acc. #043524

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100  $\mu$ g/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

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

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

**Recommended Antibody Dilutions:**

Western blotting	1:1000
Immunoprecipitation	1:50

For product specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended complementary products.

**Background References:**

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- (4) Nakae, J. et al. (1999) *J. Biol. Chem.* 274, 15982-15985.
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- (9) Yang, Y. et al. (2005) *EMBO J.* 24, 1021-1032.
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