

Phospho-FoxO3a (Ser253) Antibody

✓ 100 µl
(10 Western mini-blots)

rev. 06/08/09

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This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Entrez-Gene ID # 2309
Swiss-Prot Acc. # O43524

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

Background: The Forkhead family of transcription factors is involved in tumorigenesis in 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). There are three Akt phosphorylation sites in the FKHR proteins: Thr24, Ser256 and Ser319. Phosphorylation of FKHR family members at these sites by Akt promotes cell survival and regulates the cell cycle. Phosphorylation of FKHR proteins regulates their nuclear translocation and target gene transcription (7,8).

In FoxO3a, the three sites phosphorylated by Akt mentioned above are Thr32, Ser253 and Ser315. FoxO3a associates with 14-3-3 proteins upon phosphorylation by Akt and is retained in the cytoplasm. In the absence of survival factors, FoxO3a is dephosphorylated, translocates to the nucleus and triggers cell death by a Fas ligand-dependent mechanism (7).

Specificity/Sensitivity: Phospho-FoxO3a (Ser253) Antibody detects endogenous levels of FoxO3a only when phosphorylated at serine 253.

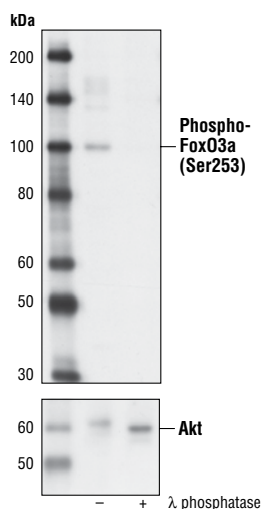
Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phospho-peptide (KLH-coupled) derived from the sequence of human FoxO3a. Antibodies are purified by protein A and peptide affinity chromatography.

Background References:

- Anderson, M.J. et al. (1998) *Genomics* 47, 187–199.
- Galili, N. et al. (1993) *Nat. Genet.* 5, 230–235.
- Borkhardt, A. et al. (1997) *Oncogene* 14, 195–202.
- Nakae, J. et al. (1999) *J. Biol. Chem.* 274, 15982–15985.
- Rena, G. et al. (1999) *J. Biol. Chem.* 274, 17179–17183.
- Guo, S. et al. (1999) *J. Biol. Chem.* 274, 17184–17192.
- Brunet, A. et al. (1999) *Cell* 96, 857–868.
- Medema, R.H. (2000) *Nature* 404, 782–787.

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.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.



Western blot analysis of extracts from PC3 cells, untreated or treated with λ phosphatase, using Phospho-FoxO3a (Ser253) Antibody (upper) or Akt Antibody #9272 (lower).

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/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 application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

