

#9946 Store at -20°C

Forkhead Signaling Antibody Sampler Kit

✓ 1 Kit
(8 x 40 µl)



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rev. 04/29/11

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.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-FoxO1 (Thr24)/FoxO3a (Thr32) Antibody	9464	40 µl	78 to 82, 95 kDa	Rabbit IgG
Phospho-FoxO1 (Ser256) Antibody	9461	40 µl	82 kDa	Rabbit IgG
Phospho-FoxO1 (Thr24)/FoxO3a (Thr32)/FoxO4 (Thr28) (4G6) Rabbit mAb	2599	40 µl	65, 78 to 82, 95	Rabbit IgG
FoxO1 (C29H4) Rabbit mAb	2880	40 µl	78 to 82 kDa	Rabbit IgG
Phospho-FoxO3a (Ser253) Antibody	9466	40 µl	97 kDa	Rabbit IgG
Phospho-FoxO3a (Ser318/321) Antibody	9465	40 µl	97 kDa	Rabbit IgG
FoxO3a (75D8) Rabbit mAb	2497	40 µl	82 to 97 kDa	Rabbit IgG
FoxO4 Antibody	9472	40 µl	65 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: This sampler kit provides an economical means to investigate Forkhead signaling. The kit contains primary and secondary antibodies to perform four Western mini-blot with each antibody.

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-β-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).

Specificity/Sensitivity:
Phospho-FoxO1 (Thr24)/FoxO3a (Thr32) Antibody detects endogenous levels of FoxO1/FoxO3a only when phosphorylated at Thr24 of FoxO1 or Thr32 of FoxO3a. The antibody cross-reacts with phosphorylated FoxO4 at Thr28.

Phospho-FoxO1 (Ser256) Antibody detects endogenous levels of FoxO1 only when phosphorylated at Ser256. The antibody cross-reacts with FoxO4 phosphorylated at Ser193.

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.

FoxO1 Antibody detects endogenous levels of FoxO1 protein and FoxO4 (at 65-70 kDa). The antibody is sensitive to phosphorylation within the antigenic site and will preferentially detect FoxO1 (or FoxO4) when not phosphorylated.

Phospho-FoxO3a (Ser253) Antibody detects endogenous levels of FoxO3a only when phosphorylated at Ser253. The antibody reacts with denatured components of bovine serum including BSA.

Phospho-FoxO3a (Ser318/321) Antibody detects endogenous levels of FoxO3a only when phosphorylated at Ser318/321. The antibody is expected to cross-react with FoxO1 when phosphorylated at Ser322/325 based on the peptide sequence.

FoxO3a (75D8) Rabbit mAb detects exogenous and endogenous levels of total FoxO3a protein. The antibody does not detect the exogenously expressed family members FoxO4 or FoxO1.

FoxO4 Antibody detects endogenous levels of FoxO4. The antibody is sensitive to phosphorylation within the antigen and preferentially detects unphosphorylated FoxO4.

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 antibodies.

Recommended Antibody Dilutions:
Western blotting 1:1000

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

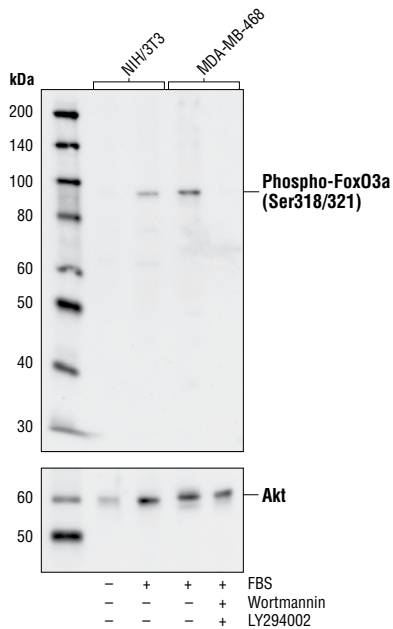
Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) derived from the sequence of human FoxO1, FoxO3a or FoxO4. Antibodies are purified by protein A and peptide affinity chromatography. Rabbit monoclonal antibodies were prepared from spleens of rabbits immunized with a synthetic peptide (KLH-coupled) corresponding to residues surrounding Glu50 of human FoxO3a or by immunizing rabbits with a GST-fusion protein corresponding to carboxy-terminal residues of human FoxO1. Antibodies are supplied in HEPES buffer with 50% glycerol and less than 0.02% sodium azide.

- Background References:**
- (1) Anderson, M.J. et al. (1998) *Genomics* 47, 187-199.
 - (2) Galili, N. et al. (1993) *Nat. Genet.* 5, 230-235.
 - (3) Borkhardt, A. et al. (1997) *Oncogene* 14, 195-202.
 - (4) Nakae, J. et al. (1999) *J. Biol. Chem.* 274, 15982-15985.
 - (5) Rena, G. et al. (1999) *J. Biol. Chem.* 274, 17179-17183.
 - (6) Guo, S. et al. (1999) *J. Biol. Chem.* 274, 17184-17192.
 - (7) Seoane, J. et al. (2004) *Cell* 117, 211-223.
 - (8) Arden, K.C. (2004) *Mol. Cell* 14, 416-418.
 - (9) Yang, Y. et al. (2005) *EMBO J.* 24, 1021-1032.

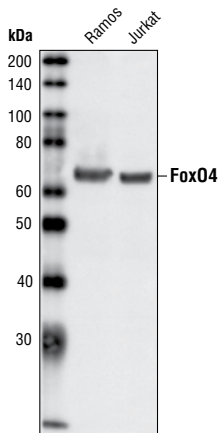
Selected rabbit monoclonal antibodies are produced under license (granting certain rights including those under U. S. Patent No. 5,675,063 and/or U.S.S.N. 11/476,277) from Epitomics, Inc. U.S.S.N. 11/476,277) from Epitomics, Inc.

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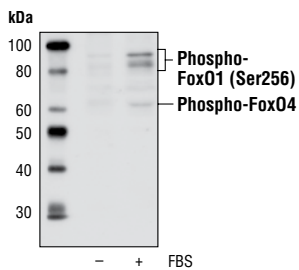
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine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



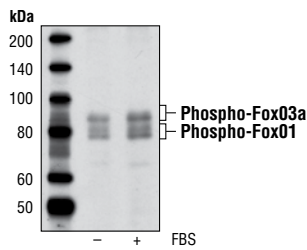
Western blot analysis of extracts from serum starved and serum-treated NIH/3T3 cells as well as untreated and LY294002/Wortmannin-treated MDA-MB-468 cells using **Phospho-FoxO3a (Ser318/321) Antibody #9465** (upper) or Akt Antibody #9272 (lower).



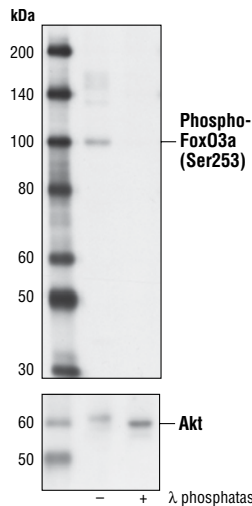
Western blot analysis of extracts from Ramos and Jurkat cells using **FoxO4 Antibody #9472**.



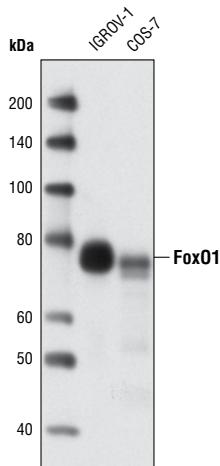
Western blot analysis of extracts from COS cells, serum starved or serum treated, using **Phospho-FoxO1 (Ser256) Antibody #9461**.



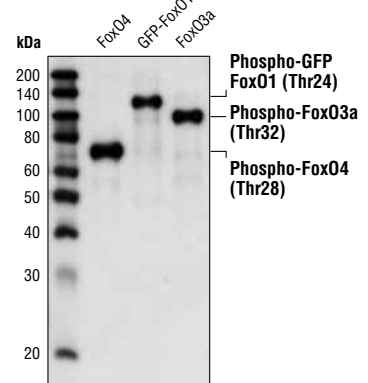
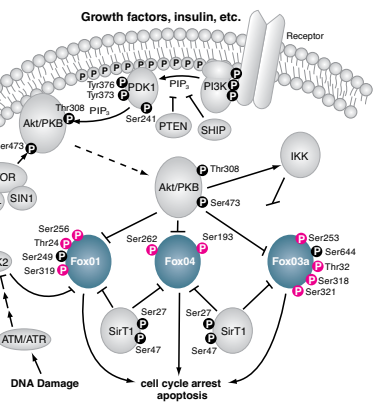
Western blot analysis of extracts from HT29 cells, serum starved or serum treated, using **Phospho-FoxO1 (Thr24)/FoxO3a (Thr32) Antibody #9464**.



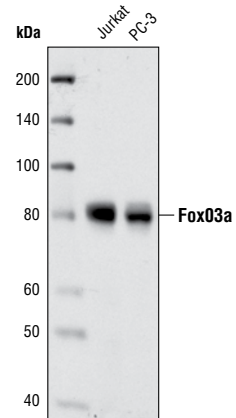
Western blot analysis of extracts from PC3 cells, untreated or treated with λ phosphatase, using **Phospho-FoxO3a (Ser253) Antibody #9466** (upper) or Akt Antibody #9272 (lower).



Western blot analysis of extracts from IGROV-1 and COS-7 cells using **FoxO1 (C29H4) Rabbit mAb #2880**.



Western blot analysis of extracts from COS-7 cells transfected with FoxO4, GFP-FoxO1 or FoxO3a using **Phospho-FoxO1 (Thr24)/FoxO3a (Thr32)/FoxO4 (Thr28) (4G6) Rabbit mAb #2599**.



Western blot analysis of extracts from Jurkat and PC-3 cells using **FoxO3a (75D8) Rabbit mAb**.

Western Immunoblotting Protocol (Primary Ab Incubation In BSA)

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.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.