Kit Includes	Quantity	Applications	Reactivity	MW (kDa)	Isotype
Phospho-FoxO1 (Thr24)/FoxO3a (Thr32) Antibody #9464	40 µl	W IP	H M R Mk	78 to 82, 95	Rabbit
Phospho-FoxO1 (Ser256) Antibody #9461	40 µl	W	H M R Mk (C) (Z)	82	Rabbit
Phospho-FoxO1 (Thr24)/FoxO3a (Thr32)/FoxO4 (Thr28) (4G6) Rabbit mAb #2599	40 µl	W	H M Mk	65, 78 to 82, 95	Rabbit IgG
FoxO1 (C29H4) Rabbit mAb #2880	40 µl	W IP IHC-P IF-IC	H M R Mk	78 to 82	Rabbit IgG
Phospho-FoxO3a (Ser253) Antibody #9466	40 µl	W IP	H M R (C)	97	Rabbit
Phospho-FoxO3a (Ser318/321) Antibody #9465	40 µl	W IP	H M R Mk (C)	97	Rabbit
FoxO3a (75D8) Rabbit mAb #2497	40 µl	W IP IF-IC	H M R Mk	82 to 97	Rabbit IgG
FoxO4 Antibody #9472	40 µl	W	H M R Mk	65	Rabbit
Anti-rabbit IgG, HRP-linked Antibody #7074	100 µl				Goat

Applications Key: W=Western Blotting IP=Immunoprecipitation IHC-P=Immunohistochemistry (Paraffin) IF-IC=Immunofluorescence (Immunocytochemistry)

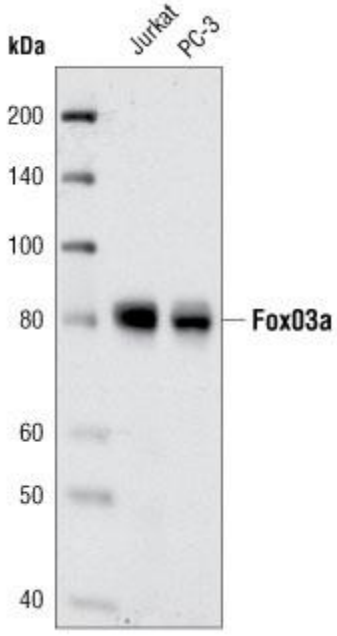
Reactivity Key: H=Human M=Mouse R=Rat Mk=Monkey C=Chicken Z=Zebrafish

Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Specificity / Sensitivity

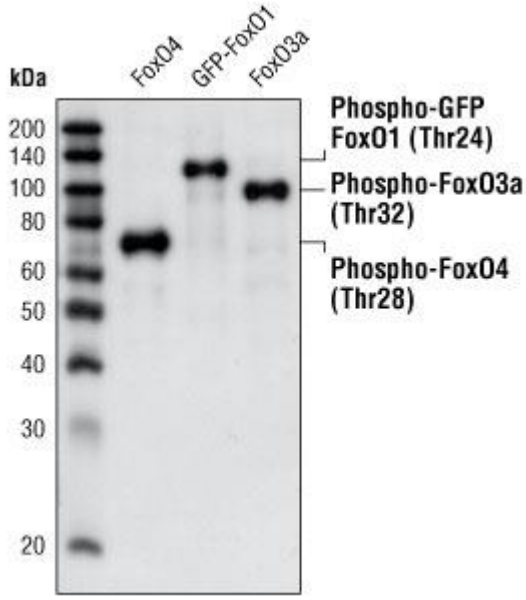
Phospho-FoxO1 (Thr24)/FoxO3a (Thr32) Antibody detects endogenous levels of FoxO1/FoxO3a only when phosphorylated at Thr24 of FoxO1 or Thr32 of FoxO3a. The antibody cross-reacts with phosphorylated FoxO4 at Thr28, but not with FoxO1 family members phosphorylated at other sites. Phospho-FoxO1 (Ser256) Antibody detects endogenous levels of FoxO1 only when phosphorylated at Ser256. The antibody cross-reacts with Fox04 phosphorylated at Ser193. Phospho-FoxO1 (Thr24)/FoxO3a (Thr32)/Fox04 (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 (C29H4) Rabbit mAb detects endogenous levels of total FoxO1 protein. The antibody does not detect exogenously expressed family members FoxO3a or FoxO4. Phospho-Fox03a (Ser253) Antibody detects endogenous levels of FoxO3a only when phosphorylated at Ser253. The antibody does not detect the transfected levels of FoxO4 or FoxO1. 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. The FoxO4 Antibody detects endogenous levels of FoxO4. The antibody is sensitive to phosphorylation within the antigen and preferrentially detects unphosphorylated FoxO4.

Western Blotting



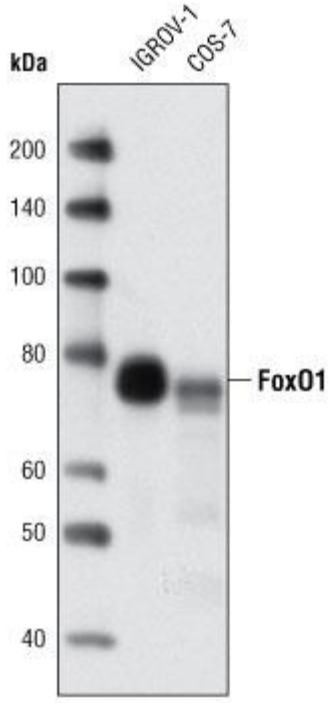
Western blot analysis of extracts from Jurkat and PC3 cells using FoxO3a (75D8) Rabbit mAb.

Western Blotting



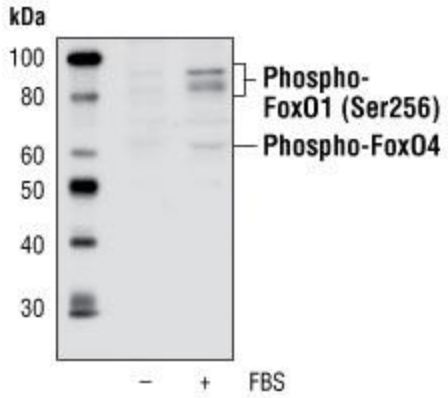
Western blot analysis of extracts from COS-7 cells transfected with FoxO4, GFP-FoxO1 or FoxO3a using Phospho-FoxO1 (Thr24)/FoxO3a (Thr32)/FoxO4 (Thr28) (4G6) Rabbit mAb #2599.

Western Blotting



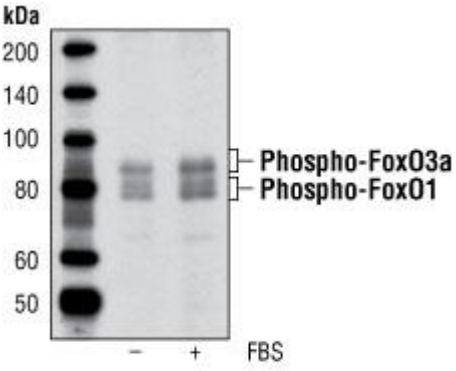
Western blot analysis of extracts from IGROV-1 and COS-7 cells using FoxO1 (C29H4) Rabbit mAb #2880.

Western Blotting



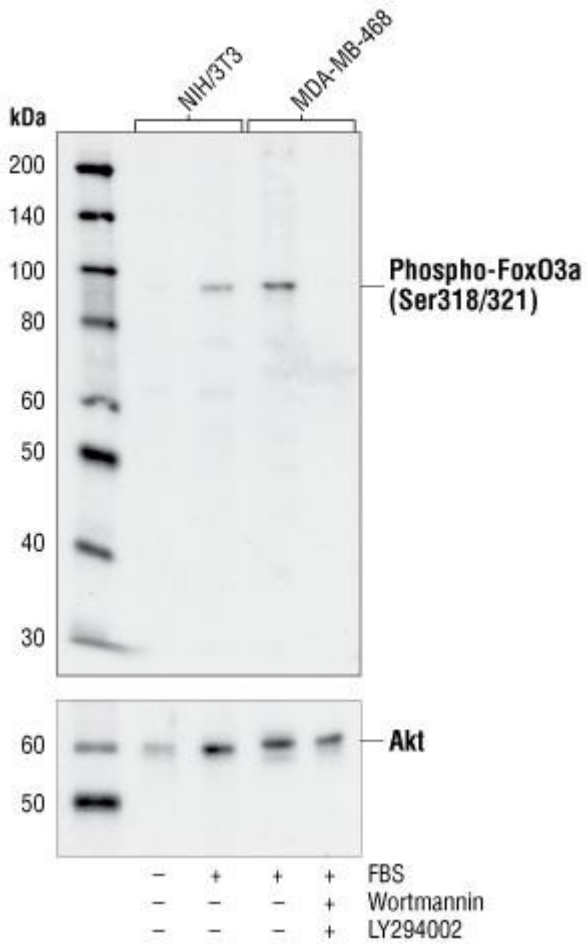
Western blot analysis of extracts from COS cells, serum starved or serum treated, using Phospho-FoxO1 (Ser256) Antibody #9461.

Western Blotting



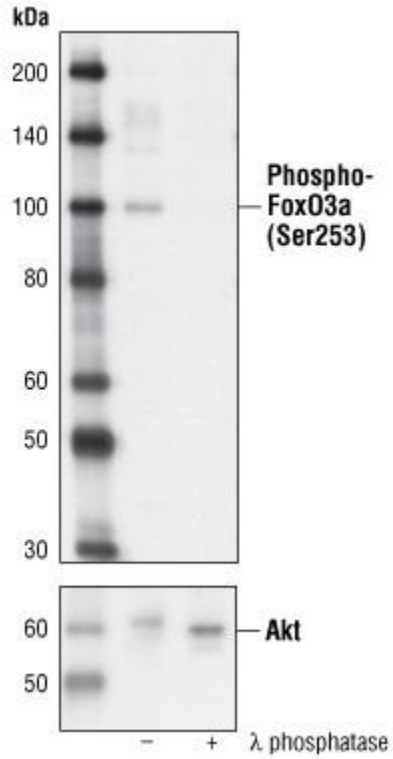
Western blot analysis of extracts from HT29 cells, serum starved or serum treated, using Phospho-FoxO1 (Thr24)/FoxO3a (Thr32) Antibody #9464.

Western Blotting



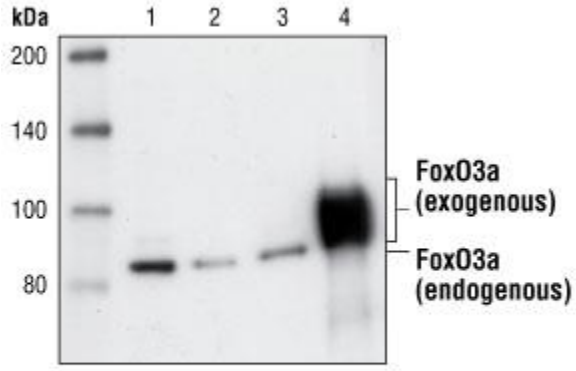
Western blot analysis of extracts from serum starved and serum-treated NIH/3T3 cells and untreated and LY294002/Wortmannin-treated MDA-MB-468 cells using Phospho-FoxO3a (Ser318/321) Antibody #9465 (upper) or Akt Antibody #9272 (lower).

Western Blotting



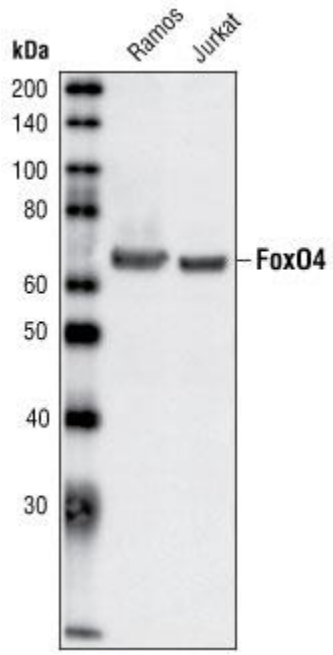
Western blot analysis of extracts from PC3 cells, untreated or treated with λ phosphatase, using Phospho-FoxO3a (Ser253) Antibody #9466 (upper) or Akt Antibody #9272 (lower).

Western Blotting



Western blot analysis of extracts from COS cells, mock transfected (lane 1), transfected with FoxO4 (lane 2), transfected with FoxO1 (lane 3), or transfected with FoxO3a (lane 4), using FoxO3a Antibody #9467.

Western Blotting



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

Description

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

Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the sequence of human FoxO1, FoxO3a or FoxO4. Antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with a synthetic peptide 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. Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Thr28 of human Fox04. Antibodies are supplied in HEPES buffer with 50% glycerol and less than 0.02% sodium azide.

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).

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