abcam

ab109629

Anti-FOXO3A antibody [EPR1950]

Recombinant | RabMAb | Advanced Validation | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR1950
Purification technique	Affinity purification Protein A
Concentration	1.506 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Not recommended

Species	Human, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000 - 1/10000
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human, Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	-
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

Target data

See full target information FOXO3

Function

Transcriptional activator that recognizes and binds to the DNA sequence 5'-[AG]TAAA[TC]A-3' and regulates different processes, such as apoptosis and autophagy (PubMed:10102273, PubMed:16751106, PubMed:21329882, PubMed:30513302). Acts as a positive regulator of autophagy in skeletal muscle: in starved cells, enters the nucleus following dephosphorylation and binds the promoters of autophagy genes, such as GABARAP1L, MAP1LC3B and ATG12, thereby activating their expression, resulting in proteolysis of skeletal muscle proteins (By similarity). Triggers apoptosis in the absence of survival factors, including neuronal cell death upon oxidative stress (PubMed:10102273, PubMed:16751106). Participates in post-transcriptional regulation of MYC: following phosphorylation by MAPKAPK5, promotes induction of miR-34b and miR-34c expression, 2 post-transcriptional regulators of MYC that bind to the 3'UTR of MYC transcript and prevent its translation (PubMed:21329882). In response to metabolic stress, translocates into the mitochondria where it promotes mtDNA transcription (PubMed:23283301). In response to metabolic stress, translocates into the mitochondria where it promotes mtDNA transcription. Also acts as a key regulator of chondrogenic commitment of skeletal progenitor cells in response to lipid availability: when lipids levels are low, translocates to the nucleus and promotes expression of SOX9, which induces chondrogenic commitment and suppresses fatty acid oxidation (By similarity). Also acts as a key regulator of regulatory T-cells (Treg) differentiation by activating expression of FOXP3 (PubMed:30513302).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage conditions	+4°C

Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Stable for 12 months at -20°C

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	FOXO3A also known as FOXO3 or 1e2 is a transcription factor with a molecular weight of approximately 71 kDa. It belongs to the Forkhead box O family of proteins. This protein plays an important role in the regulation of transcription in response to oxidative stress and growth stimuli. FOXO3A is expressed in a variety of tissues including liver muscle and brain where it contributes to a wide range of cellular processes related to stress resistance and metabolism. Scientists frequently study FOXO3A using techniques like FOXO3A ELISA and apply FOXO3A anticuerpos in research to understand its function more intricately.
Biological function summary	FOXO3A acts as a regulator of genes involved in cell cycle arrest apoptosis and DNA repair. It is not part of a simple protein complex but works closely with multiple factors to influence cellular homeostasis. Significantly FOXO3A controls the expression of Bcl-2 a protein important for cell survival and the protein p27 which is important for cell cycle control. FOXO3A's activity modulates cellular longevity and impacts stress responses highlighting its abundant biological functions.
Pathways	FOXO3A integrates into the PI3K/AKT and the insulin signaling pathways both essential for cellular metabolism and proliferation control. In the PI3K/AKT pathway FOXO3A functionally links with proteins such as AKT and PTEN where its activity can be inhibited by AKT-mediated phosphorylation. In doing so it supports a balance between cell survival and apoptosis allowing the cell to adjust to growth conditions and external stressors effectively.

Associated diseases and disorders	FOXO3A is linked to cancer and neurodegenerative diseases. In cancer alterations in FOXO3A activity or expression can affect tumor growth and resistance to oxidative stress often involving interactions with proteins like MDM2. Similarly in neurodegenerative disorders such as Alzheimer's disease FOXO3A modifies responses to oxidative damage potentially tied to proteins like Tau. Both instances highlight the role of FOXO3A as a versatile modulator that can influence the progression and management of various diseases.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

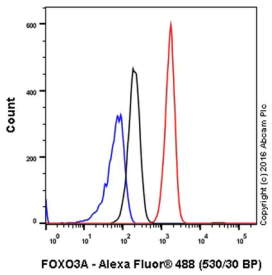
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

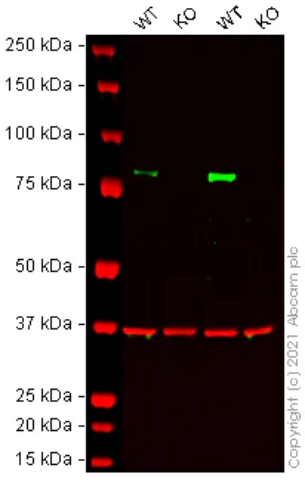
7 product images



Flow Cytometry (Intracellular) - Anti-FOXO3A antibody [EPR1950] (ab109629)

FOXO3A Flow Cytometry (Intracellular) staining using rabbit Anti-FOXO3A antibody

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling FOXO3A with unpurified ab109629 at 1/150 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Western blot - Anti-FOXO3A antibody [EPR1950] (ab109629)

FOXO3A Western blot staining using rabbit Anti-FOXO3A antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab109629 observed at 82 kDa. Red - loading control ab8245 (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.
ab109629 was shown to react with FOXO3A in wild-type cells in Western blot with loss of signal observed in FOXO3 knockout cell lines. Wild-type and FOXO3 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab109629 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.

All lanes:
Western blot - Anti-FOXO3A antibody [EPR1950] (ab109629) at 1/1000 dilution

Lane 1:
Wild-type HEK-293T cell lysate at 20 µg

Lane 2:
Western blot - Human FOXO3 (FOXO3A) knockout HEK-293T cell lysate (ab256922) at 20 µg

Lane 2:
Western blot - Human FOXO3 (FOXO3A) knockout HEK-293T cell line (ab265069)

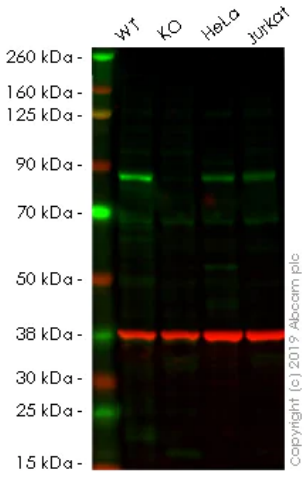
Lane 3:
Wild-type HEK-293 cell lysate at 20 µg

Lane 4:
Western blot - Human FOXO3 (FOXO3A) knockout HEK-293 cell lysate (ab261649) at 20 µg

Performed under reducing conditions.

Predicted band size: 71 kDa

Observed band size: 82 kDa



Western blot - Anti-FOXO3A antibody [EPR1950] (ab109629)

FOXO3A Western blot staining using rabbit Anti-FOXO3A antibody

Lanes 1 - 4: Merged signal (red and green). Green - ab109629 observed at 71 kDa. Red - loading control, ab8245, observed at 37 kDa.
ab109629 was shown to recognize FOXO3A in wild-type HEK 293 cells as signal was lost at the expected MW in FOXO3 knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and FOXO3 knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% Milk. ab109629 and ab8245 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-FOXO3A antibody [EPR1950] (ab109629) at 1/1000 dilution

Lane 1:
Wild-type HEK 293 whole cell lysate at 20 µg

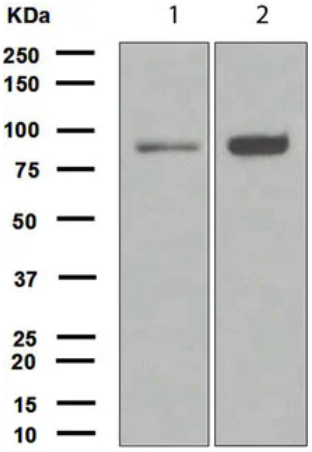
Lane 2:
FOXO3 knockout HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate at 20 µg

Lane 2:
Western blot - Human FOXO3 (FOXO3A) knockout HEK-293 cell line (ab260857)

Lane 3:
HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

Lane 4:
Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate at 20 µg

Predicted band size: 71 kDa



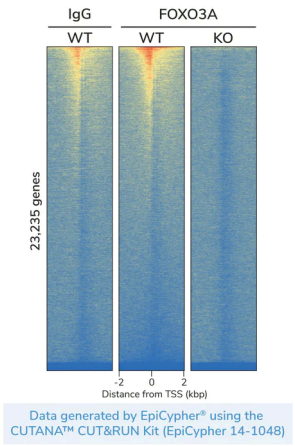
Western blot - Anti-FOXO3A antibody [EPR1950] (ab109629)

All lanes:
Western blot - Anti-FOXO3A antibody [EPR1950] (ab109629) at 1/1000 dilution

Lane 1:
MCF7 cell lysate at 10 µg

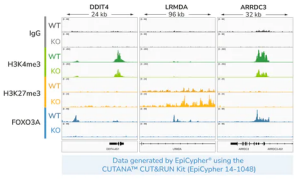
Lane 2:
SH-SY5Y cell lysate at 10 µg

Predicted band size: 71 kDa



ChIC/CUT&RUN sequencing - Anti-FOXO3A antibody [EPR1950] (ab109629)

CUT&RUN profiling with FOXO3A antibody demonstrates robust genome-wide enrichment in wild-type (WT) cells, which is markedly diminished in FOXO3 knockout (KO) cells. Heatmaps of genome-wide signal flanking annotated transcription start sites (TSSs, +/- 2 kbp) display CUT&RUN data generated using the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048) with FOXO3A antibody (Abcam ab109629, 0.5 µg). 500,000 HEK293T WT or KO (Abcam ab265069) cells were used per reaction. IgG antibody was included as a negative control to assess non-specific background. Libraries were prepared using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001). Sequencing was performed with paired-end 50 bp reads, and data were processed on CUTANA™ Cloud (cloud.epicypher.com) by alignment to the hg38 genome. Heatmaps were generated using deepTools (Ramнrez et al., Nucleic Acids Res. 2014; PMID: 24799436). Row-linked data are ranked by intensity relative to FOXO3A WT, with red indicating high localized enrichment and blue denoting background. Validated antibodies show genome-wide enrichment above IgG background consistent with FOXO3A binding in WT cells and near complete loss of signal in KO cells.



ChIC/CUT&RUN sequencing - Anti-FOXO3A antibody [EPR1950] (ab109629)

CUT&RUN profiling with FOXO3A antibody reveals the expected genomic enrichment pattern in wild-type (WT) cells, which is substantially reduced in FOXO3 knockout (KO) cells. Representative genome browser tracks show CUT&RUN data generated using the CUTANA™ CUT&RUN Kit (EpiCypher 14-1048) with FOXO3A antibody (Abcam ab109629, 0.5 µg). 500,000 HEK293T WT or KO (Abcam ab265069) cells were used per reaction. IgG, H3K4me3, and H3K27me3 antibodies were included as controls to assess non-specific background, active promoters, and repressed chromatin, respectively. Libraries were prepared using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001). Sequencing was performed with paired-end 50 bp reads, and data were processed on CUTANA™ Cloud (cloud.epicypher.com) by alignment to the hg38 genome. Images were generated using Integrative Genomics Viewer (IGV, Broad Institute).

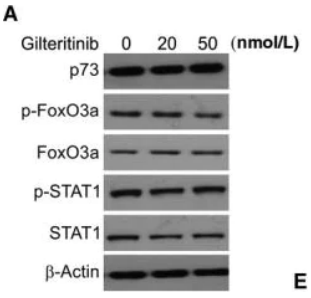


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Western blot - Anti-FOXO3A antibody [EPR1950] (ab109629)

FOXO3A western blot using anti-FOXO3A antibody [EPR1950] ab109629. Publication image and figure legend from Li, L., Lin, L., et al., 2020, J Cell Mol Med, PubMed 31881122.

ab109629 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab109629 please see the product overview.

Activation of p65 mediates PUMA induction in response to gilteritinib treatment. A, HCT116 cells were treated with gilteritinib for 24 h at indicated concentration. Indicated proteins were analysed by Western blot. B, HCT116 cells were treated with 50 nmol/L gilteritinib at indicated time□points. Expression of p□p65 (S536) and p65 was analysed by Western blot. C, HCT116 cells were transfected with either a control scrambled siRNA or a p65 siRNA for 24 h and then treated with 50 nmol/L gilteritinib for 24 h. p65 and PUMA expressions were analysed by Western blot. D, HCT116 cells were treated with 10 µmol/L BAY11□7082 for 1 h, and then with 50 nmol/L gilteritinib for 24 h. Nuclear fractions were isolated from cells and analysed for p65 expression by Western blot. Lamin A/C and β□actin, which are expressed in nucleus and cytoplasm, respectively, were used as controls for loading and fractionation. E, HCT116 cells were treated with 10 µmol/L BAY11□7082 for 1 h, and then with 50 nmol/L gilteritinib for 24 h. The level of p□p65 (S536) and PUMA was analysed by Western blot. F, Chromatin immunoprecipitation (ChIP) was performed using anti□p65 antibody on HCT116 cells following gilteritinib (50 nmol/L) treatment for 12 h. The IgG was used to control for antibody specificity. PCR was carried out using primers surrounding the p65 binding sites in the PUMA promoter

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