abcam

Product datasheet

Anti-FOXO3A antibody [EPR1950] ab109629

KO VALIDATED Recombinant RabMAb

1 Abreviews 26 References 5 Images

Overview

Product name	Anti-FOXO3A antibody [EPR1950]
Description	Rabbit monoclonal [EPR1950] to FOXO3A
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB **Unsuitable for:** IHC-P or IP
Species reactivity	**Reacts with:** Human **Predicted to work with:** Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	HEK-293, HEK-293T, MCF7 and SH-SY5Y cell lysates, HeLa cells
General notes	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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture supernatant
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR1950

Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab109629 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

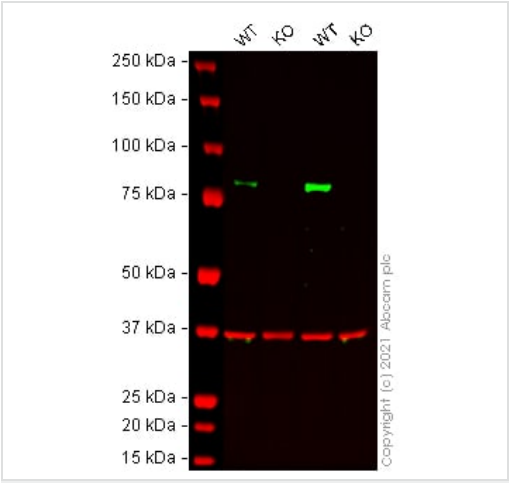
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		1/1000 - 1/10000. Detects a band of approximately 90 kDa (predicted molecular weight: 71 kDa).

Application notes Is unsuitable for IHC-P or IP.

Target

Function	Transcriptional activator which triggers apoptosis in the absence of survival factors, including neuronal cell death upon oxidative stress. Recognizes and binds to the DNA sequence 5'-[AG]TAAA[TC]A-3'.
Tissue specificity	Ubiquitous.
Involvement in disease	Note=A chromosomal aberration involving FOXO3 is found in secondary acute leukemias. Translocation t(6;11)(q21;q23) with MLL/HRX.
Sequence similarities	Contains 1 fork-head DNA-binding domain.
Post-translational modifications	In the presence of survival factors such as IGF-1, phosphorylated on Thr-32 and Ser-253 by AKT1/PKB. This phosphorylated form then interacts with 14-3-3 proteins and is retained in the cytoplasm. Survival factor withdrawal induces dephosphorylation and promotes translocation to the nucleus where the dephosphorylated protein induces transcription of target genes and triggers apoptosis. Although AKT1/PKB doesn't appear to phosphorylate Ser-315 directly, it may activate other kinases that trigger phosphorylation at this residue. Phosphorylated by STK4 on Ser-209 upon oxidative stress, which leads to dissociation from YWHAB/14-3-3-beta and nuclear translocation. Phosphorylated by PIM1.
Cellular localization	Cytoplasm > cytosol. Nucleus. Translocates to the nucleus upon oxidative stress and in the absence of survival factors.

Images



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

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

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : Human FOXO3 (FOXO3A) knockout HEK-293T cell lysate (**ab256922**)
Lane 3 : Wild-type HEK-293 cell lysate
Lane 4 : Human FOXO3 (FOXO3A) knockout HEK-293 cell lysate (**ab261649**)

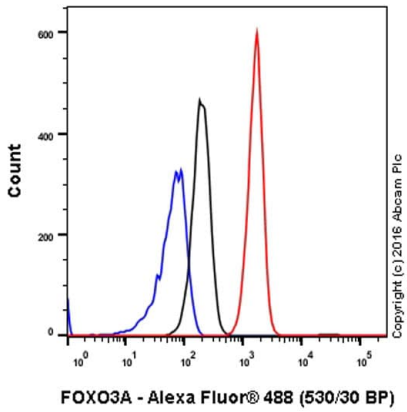
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 71 kDa
Observed band size: 82 kDa

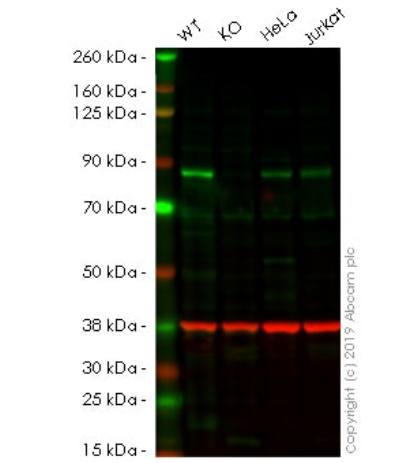
Lanes 1 - 4: Merged signal (red and green). Green - ab109629 observed at 82 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

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.



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

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)

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

Lane 1 : Wild-type HEK 293 whole cell lysate
Lane 2 : FOXO3 knockout HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

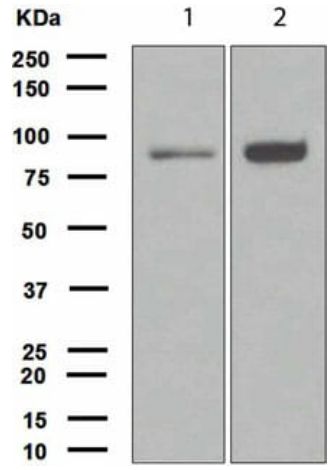
Lysates/proteins at 20 µg per lane.

Predicted band size: 71 kDa

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.



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

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

Lane 1 : MCF7 cell lysate
Lane 2 : SH-SY5Y cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 71 kDa

Anti-FOXO3A antibody [EPR1950] (ab109629)

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish

- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors