abcam

Product datasheet

Anti-FOXO3A antibody [EPR1950] ab109629





重组 RabMAb

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概述

产品名称 Anti-FOXO3A抗体[EPR1950]

描述 兔单克隆抗体[EPR1950] to FOXO3A

宿主 Rabbit

经测试应用 适用于: Flow Cyt (Intra), WB

不适用于: IHC-P or IP

种属反应性 与反应: Human

预测**可用于:** Rat 4

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

阳性对照 HEK-293, HEK-293T, MCF7 and SH-SY5Y cell lysates, HeLa cells

常规说明 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.

性能

形式 Liquid

存放说明 Shipped at 4°C. Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.

存储溶液 pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture

supernatant

纯度 Protein A purified

克隆 单克隆 克隆编号 **EPR1950**

同种型 lgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab109629于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
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).

应用说明 Is unsuitable for IHC-P or IP.

靶标

功能 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'.

组织特异性 Ubiquitous.

疾病相关 Note=A chromosomal aberration involving FOXO3 is found in secondary acute leukemias.

Translocation t(6;11)(q21;q23) with MLL/HRX.

序列相似性 Contains 1 fork-head DNA-binding domain.

翻译后修饰 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

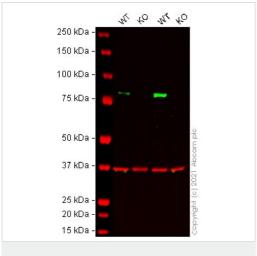
upon oxidative stress, which leads to dissociation from YWHAB/14-3-3-beta and nuclear

translocation. Phosphorylated by PIM1.

细胞定位 Cytoplasm > cytosol. Nucleus. Translocates to the nucleus upon oxidative stress and in the

absence of survival factors.

图片



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)

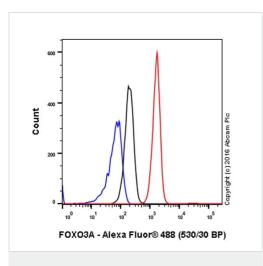
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

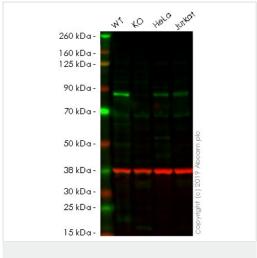
Lanes 1 - 4: Merged signal (red and green). Green - ab109629 observed at 82 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

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 lgG H&L (IRDye[®] 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG 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, <u>ab8245</u>, 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 <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

KDa 1 2

250 — 150 — 100 — 75 — 50 — 37 — 25 — 20 — 15 — 10 — 15 — 10 — 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



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