

Anti-FOXO3A antibody ab23683

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概述

产品名称	Anti-FOXO3A抗体
描述	兔多克隆抗体to FOXO3A
宿主	Rabbit
特异性	Replenishment batches of our polyclonal antibody, ab23683 are tested in WB. Previous batches were additionally validated in ICC/IF and IHC-P. These applications are still expected to work and are covered by our Abpromise guarantee. You may also be interested in our alternative recombinant antibody, ab109629 .
经测试应用	适用于: WB, IHC-P, ICC/IF
种属反应性	与反应: Mouse, Rat, Human 预测可用于: Dog 
免疫原	Synthetic peptide corresponding to Human FOXO3A aa 650 to the C-terminus conjugated to keyhole limpet haemocyanin. (Peptide available as ab24031)
阳性对照	Recombinant Human FOXO3A protein (ab114191) can be used as a positive control in WB. This antibody gave a positive signal in the following whole cell lysates: Jurkat (Human T cell lymphoblast-like cell line) This antibody gave a positive signal in the following tissue lysates: Mouse Heart Mouse Skeletal Muscle Rat Heart This antibody gave a positive result in IHC in the following FFPE tissue: Human normal lung.
常规说明	<p>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</p> <p>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As</p>

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.

存储溶液 pH: 7.40
Preservative: 0.02% Sodium azide
Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

纯度 Immunogen affinity purified

克隆 多克隆

同种型 IgG

应用

The Abpromise guarantee **Abpromise™** 承诺保证使用ab23683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
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 90 kDa (predicted molecular weight: 71 kDa).
IHC-P		Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		Use a concentration of 5 µg/ml.

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.

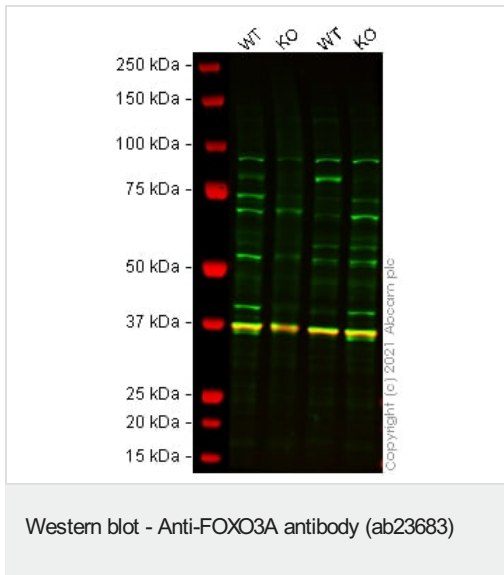
疾病相关 Note=A chromosomal aberration involving FOXO3 is found in secondary acute leukemias. Translocation t(6;11)(q21;q23) with MLL/HRX.

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



All lanes : Anti-FOXO3A antibody (ab23683) at 1 µg/ml

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.

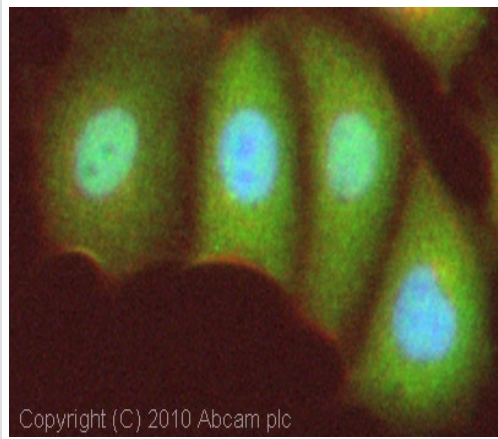
Performed under reducing conditions.

Predicted band size: 71 kDa

Observed band size: 82 kDa

Lanes 1 -4: Merged signal (red and green). Green - ab23683 observed at 82 kDa. Red - loading control [ab8245](#) (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab23683 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 ab23683 and [ab8245](#) (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at 1 µg/ml 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.



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Immunocytochemistry/ Immunofluorescence - Anti-FOXO3A antibody (ab23683)

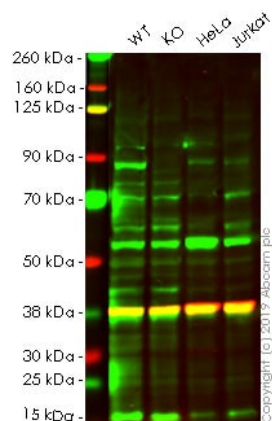
ICC/IF image of ab23683 stained MCF7 cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab23683, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM. This antibody also gave a positive result in 4% HepG2 fixed (10 min) HepG2 cells at 5µg/ml.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-FOXO3A antibody (ab23683)

IHC image of FOXO3A staining in Human normal lung formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab23683, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Western blot - Anti-FOXO3A antibody (ab23683)

All lanes : Anti-FOXO3A antibody (ab23683) at 1 µg/ml

Lane 1 : Wild-type HEK-293 (Human epithelial cell line from embryonic kidney) 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.

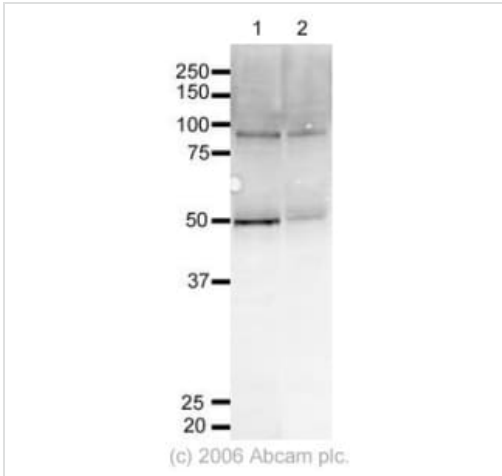
Performed under reducing conditions.

Predicted band size: 71 kDa

Observed band size: 82 kDa

Lanes 1 -4: Merged signal (red and green). Green - ab23683 observed at 71 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab23683 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. Ab23683 and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/ml 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 (ab23683)

All lanes : Anti-FOXO3A antibody (ab23683) at 1 µg/ml

Lane 1 : Jurkat whole cell lysate (**ab7899**)

Lane 2 : Jurkat whole cell lysate (**ab7899**) with Human FOXO3A peptide (**ab24031**) at 1 µg/ml

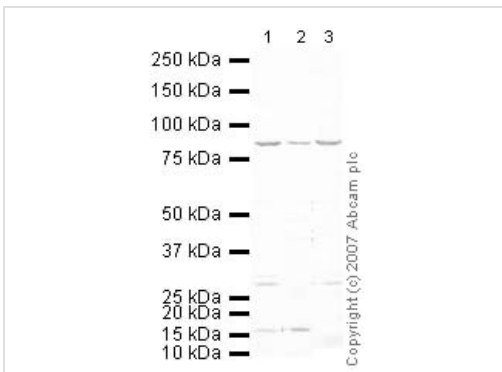
Lysates/proteins at 20 µg per lane.

Predicted band size: 71 kDa

Observed band size: 90 kDa

Additional bands at: 50 kDa (possible cleavage fragment), 50 kDa (possible cross reactivity)

ab23683 detects a band at approximately 90 kDa that corresponds in size to that seen for FOXO3A. This protein has been shown to migrate at a size larger than the predicted molecular weight (see Yin et al., J. Biol. Chem., Vol. 279, Issue 44, 45721-45727). It also detects a band at 50 kDa which could be a cleavage fragment. Both bands are partially blocked by the addition of the immunizing peptide.



Western blot - Anti-FOXO3A antibody (ab23683)

All lanes : Anti-FOXO3A antibody (ab23683) at 1 µg/ml

Lane 1 : Heart (Mouse) Tissue Lysate - normal tissue

Lane 2 : Mouse skeletal muscle tissue lysate - total protein (**ab29711**)

Lane 3 : Heart (Rat) Tissue Lysate - normal tissue

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

Predicted band size: 71 kDa

Observed band size: 90 kDa

Additional bands at: 16 kDa, 27 kDa. We are unsure as to the identity of these extra bands.

ab23683 detects a band at approximately 90 kDa that corresponds in size to that seen for FOXO3A. This protein has been shown to migrate at a size larger than the predicted molecular weight (see Yin et al., J. Biol. Chem., Vol. 279, Issue 44, 45721-45727).

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