

Product datasheet

Anti-FOXO1A (pT24)+FOXO3A (pT32)+FOXO4 (pT28)+FOXO6 (pT26) antibody [EPR28359-78]-BSA and Azide free ab312327

Recombinant RabMAb

8 Images

Overview

Product name	Anti-FOXO1A (pT24)+FOXO3A (pT32)+FOXO4 (pT28)+FOXO6 (pT26) antibody [EPR28359-78]-BSA and Azide free
Description	Rabbit monoclonal [EPR28359-78] to FOXO1A (phospho T24) + FOXO4/AFX (phospho T28) + FOXO3A (phospho T32) + Foxo6 (phospho T26) - BSA and Azide free
Host species	Rabbit
Tested applications	Suitable for: WB, Dot blot, Flow Cyt (Intra) Unsuitable for: ICC/IF, IHC-P or IP
Species reactivity	Reacts with: Mouse, Human Does not react with: Rat
Immunogen	This product was produced with the following immunogens: Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: NIH/3T3 treated with 100nM Calycin A, Jurkat starved overnight, then treated with 20% serum and 100nM Calycin A, 293T transfected with mouse FOXO1A, 293T transfected with mouse FOXO3A, 293T transfected with mouse FOXO4/AFX and 293T transfected with mouse FOXO6 (WT) expression vector containing a myc-His-tag®, then treated with 100nM Calyculin A Flow Cyt (intra): NIH/3T3 treated with 100nM Calyculin A Dot: FOXO1A (phospho T24), FOXO3 (phospho T32), FOXO4 (phospho T28), FOXO6 (phospho T26) peptide
General notes	ab312327 is the carrier-free version of ab312326 . Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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. Store at +4°C.
Storage buffer	pH: 7.2 Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR28359-78
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab312327 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
WB		Use at an assay dependent concentration. Predicted molecular weight: 69 kDa.
Dot blot		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.

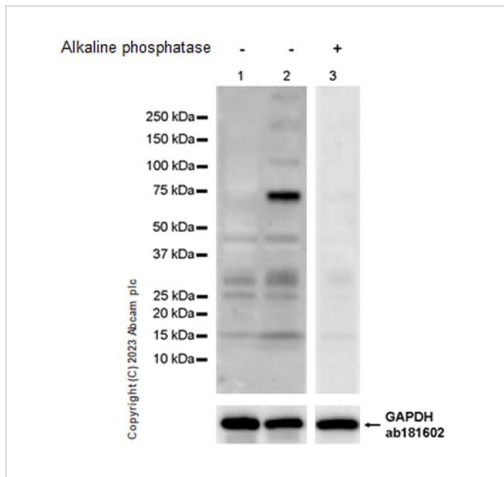
Application notes Is unsuitable for ICC/IF, IHC-P or IP.

Target

Cellular localization FOXO1A: Cytoplasm. Nucleus. Shuttles between cytoplasm and nucleus. FOXO4/AFX:

Cytoplasm. Nucleus. When phosphorylated, translocated from nucleus to cytoplasm. Dephosphorylation triggers nuclear translocation. Monoubiquitination increases nuclear localization. When deubiquitinated, translocated from nucleus to cytoplasm. FOXO3A: Cytoplasm > cytosol. Nucleus. Translocates to the nucleus upon oxidative stress and in the absence of survival factors. Foxo6: Cytoplasmic and Nuclear. When phosphorylated, translocated from nucleus to cytoplasm. High nuclear localization after stimulation with growth factors.

Images



Western blot - Anti-FOXO1A (phospho T24) + FOXO3A (phospho T32) + FOXO4/AFX (phospho T28) + FOXO6 (phospho T26) antibody [EPR28359-78] - BSA and Azide free (ab312327)

All lanes : Anti-FOXO1A (pT24)+FOXO3A (pT32)+FOXO4 (pT28)+FOXO6 (pT26) antibody [EPR28359-78] ([ab312326](#)) at 1/1000 dilution

Lane 1 : Untreated NIH/3T3 (mouse embryonic fibroblast) whole cell lysate (untreated membrane)

Lane 2 : NIH/3T3 treated with 100nM Calyculin A for 30min whole cell lysate (untreated membrane)

Lane 3 : NIH/3T3 treated with 100nM Calyculin A for 30min whole cell lysate (phosphatase treated membrane)

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Predicted band size: 69 kDa

Observed band size: 75 kDa

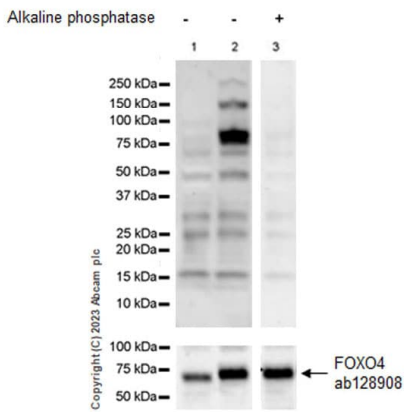
Exposure time: 81 seconds

This data was developed using [ab312326](#), the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFD/MTBST

In Western blot, anti-GAPDH antibody ([ab181602](#)) loading control staining at 1/200000 dilution.

Exposure time: 81 seconds.



Western blot - Anti-FOXO1A (phospho T24) + FOXO3A (phospho T32) + FOXO4/AFX (phospho T28) + FOXO6 (phospho T26) antibody [EPR28359-78] - BSA and Azide free (ab312327)

All lanes : Anti-FOXO1A (pT24)+FOXO3A (pT32)+FOXO4 (pT28)+FOXO6 (pT26) antibody [EPR28359-78] (**ab312326**) at 1/1000 dilution

Lane 1 : Jurkat (human t cell leukemia t lymphocyte from peripheral blood) starved overnight, then treated with 50 uM LY294092 for 30min whole cell lysate (untreated membrane)

Lane 2 : Jurkat starved overnight, then treated with 20% serum and 100nM Calycin A for 30min whole cell lysate (untreated membrane)

Lane 3 : Jurkat starved overnight, then treated with 20% serum and 100nM Calycin A for 30min whole cell lysate (phosphatase treated membrane)

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 69 kDa

Observed band size: 75-80 kDa

Exposure time: 59 seconds

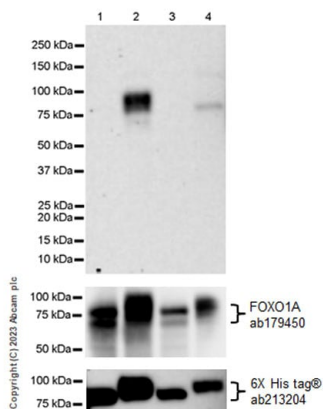
This data was developed using **ab312326**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDm/TBST

The bands above 100 kDa are unknown.

In Western blot, anti-FOXO4 antibody (**ab128908**) staining at 1/1000 dilution.

Exposure time: 59 seconds.



Western blot - Anti-FOXO1A (phospho T24) + FOXO3A (phospho T32) + FOXO4/AFX (phospho T28) + FOXO6 (phospho T26) antibody [EPR28359-78] - BSA and Azide free (ab312327)

All lanes : Anti-FOXO1A (pT24)+FOXO3A (pT32)+FOXO4 (pT28)+FOXO6 (pT26) antibody [EPR28359-78] ([ab312326](#)) at 1/1000 dilution

Lane 1 : Untreated 293T (human embryonic kidney epithelial cell) transfected with mouse FOXO1A (WT) expression vector contain a myc-His-tag® whole cell lysate

Lane 2 : 293T transfected with mouse FOXO1A (WT) expression vector contain a myc-His-tag®, then treated with 100nM Calyculin A for 30min whole cell lysate

Lane 3 : Untreated 293T transfected with mouse FOXO1A (Mutant T24A) expression vector contain a myc-His-tag® whole cell lysate

Lane 4 : 293T transfected with mouse FOXO1A (Mutant T24A) expression vector contain a myc-His-tag®, then treated with 100nM Calyculin A for 30min whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

Predicted band size: 69 kDa

Observed band size: 78-82 kDa

Exposure time: 3 minutes

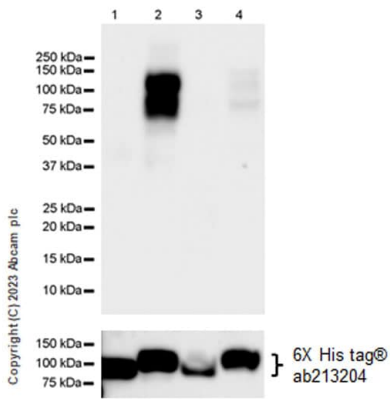
This data was developed using [ab312326](#), the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDM/TBST

In Western blot, anti-FOXO1A antibody ([ab179450](#)) staining at 1/1000 dilution.

In Western blot, anti-His antibody ([ab213204](#)) staining at 1/5000 dilution.

Exposure time: 3 minutes.



Western blot - Anti-FOXO1A (phospho T24) + FOXO3A (phospho T32) + FOXO4/AFX (phospho T28) + FOXO6 (phospho T26) antibody [EPR28359-78] - BSA and Azide free (ab312327)

All lanes : Anti-FOXO1A (pT24)+FOXO3A (pT32)+FOXO4 (pT28)+FOXO6 (pT26) antibody [EPR28359-78] (**ab312326**) at 1/1000 dilution

Lane 1 : Untreated 293T (human embryonic kidney epithelial cell) transfected with mouse FOXO3A (WT) expression vector containi a myc-His-tag® whole cell lysate

Lane 2 : 293T transfected with mouse FOXO3A (WT) expression vector containi a myc-His-tag®, then treated with 100nM Calyculin A for 30min whole cell lysate

Lane 3 : Untreated 293T transfected with mouse FOXO3A (Mutant T32A) expression vector containi a myc-His-tag® whole cell lysate

Lane 4 : 293T transfected with mouse FOXO3A (Mutant T32A) expression vector containi a myc-His-tag®, then treated with 100nM Calyculin A for 30min whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 69 kDa

Observed band size: 78-140 kDa

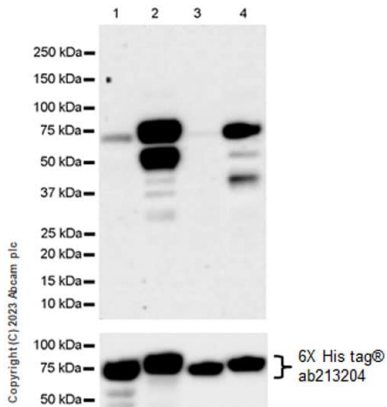
Exposure time: 6 seconds

This data was developed using **ab312326**, the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDN/TBST

In Western blot, anti-His antibody (**ab213204**) staining at 1/5000 dilution.

Exposure time: 6 seconds.



Western blot - Anti-FOXO1A (phospho T24) + FOXO3A (phospho T32) + FOXO4/AFX (phospho T28) + FOXO6 (phospho T26) antibody [EPR28359-78] - BSA and Azide free (ab312327)

All lanes : Anti-FOXO1A (pT24)+FOXO3A (pT32)+FOXO4 (pT28)+FOXO6 (pT26) antibody [EPR28359-78] ([ab312326](#)) at 1/1000 dilution

Lane 1 : Untreated 293T (human embryonic kidney epithelial cell) transfected with mouse FOXO4/AFX (WT) expression vector containi a myc-His-tag® whole cell lysate

Lane 2 : 293T transfected with mouse FOXO4/AFX (WT) expression vector containi a myc-His-tag®, then treated with 100nM Calyculin A for 30min whole cell lysate

Lane 3 : Untreated 293T transfected with mouse FOXO6 (WT) expression vector containi a myc-His-tag® whole cell lysate

Lane 4 : 293T transfected with mouse FOXO6 (WT) expression vector containi a myc-His-tag®, then treated with 100nM Calyculin A for 30min whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

Predicted band size: 69 kDa

Observed band size: 50, 75 kDa

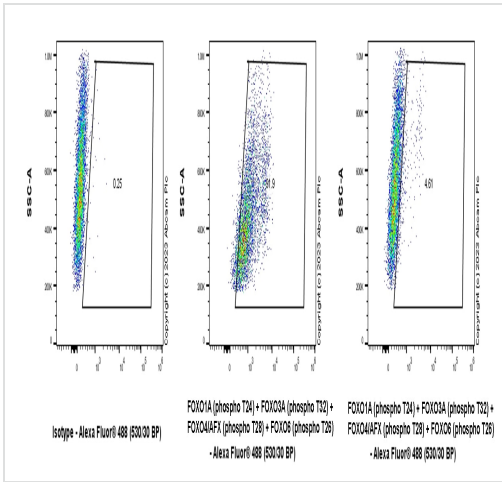
Exposure time: 180 seconds

This data was developed using [ab312326](#), the same antibody clone in a different buffer formulation.

Blocking and diluting buffer and concentration: 5% NFDm/TBST

In Western blot, anti-His antibody ([ab213204](#)) staining at 1/5000 dilution.

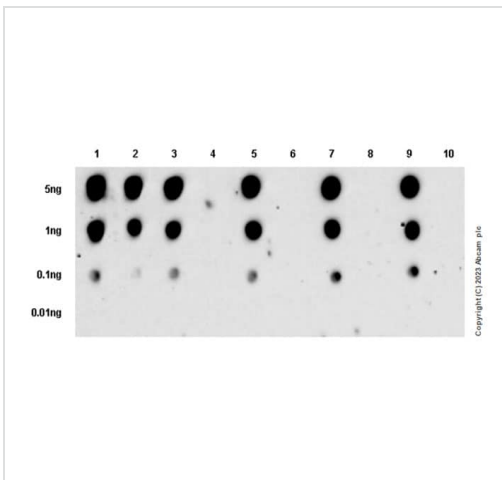
Exposure time: 180 seconds.



Flow Cytometry (Intracellular) - Anti-FOXO1A (phospho T24) + FOXO3A (phospho T32) + FOXO4/AFX (phospho T28) + FOXO6 (phospho T26) antibody [EPR28359-78] - BSA and Azide free (ab312327)

This data was developed using [ab312326](#), the same antibody clone in a different buffer formulation.

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Isotype (Left) / NIH/3T3 (mouse embryonic fibroblast) treated with 100nM Calyculin A for 30min (Middle) / Untreated NIH/3T3 (Right) cells labelling FOXO1A (phospho T24) + FOXO3A (phospho T32) + FOXO4/AFX (phospho T28) + FOXO6 (phospho T26) with [ab312326](#) at 1/500 dilution (0.1 ug)/Right (Red) compared with a Rabbit monoclonal IgG ([ab172730](#)) / Left isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, [ab150081](#)) at 1/5000 dilution was used as the secondary antibody.



Dot Blot - Anti-FOXO1A (phospho T24) + FOXO3A (phospho T32) + FOXO4/AFX (phospho T28) + FOXO6 (phospho T26) antibody [EPR28359-78] - BSA and Azide free (ab312327)

This data was developed using [ab312326](#), the same antibody clone in a different buffer formulation.

Dot blot analysis of FOXO1A (phospho T24) + FOXO3A (phospho T32) + FOXO4/AFX (phospho T28) + FOXO6 (phospho T26) using [ab312326](#) at 1:1000 (0.5 ug/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated ([ab97051](#)) at 1:100,000 dilution.

- Lane 1: FOXO1A (phospho T24) peptide 1
- Lane 2: FOXO1A (phospho T24) peptide 2
- Lane 3: FOXO1A (phospho T24) peptide 3
- Lane 4: FOXO1A non-phospho peptide
- Lane 5: FOXO3 (phospho T32) peptide
- Lane 6: FOXO3 non-phospho peptide
- Lane 7: FOXO4 (phospho T28) peptide
- Lane 8: FOXO4 non-phospho peptide
- Lane 9: FOXO6 (phospho T26) peptide
- Lane 10: FOXO6 non-phospho peptide

Exposure time: 180 seconds

Blocking and diluting buffer and concentration: 5% NFD/MTBST

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-FOXO1A (phospho T24) + FOXO3A (phospho T32) + FOXO4/AFX (phospho T28) + FOXO6 (phospho T26) antibody [EPR28359-78] - BSA and Azide free (ab312327)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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