abcam

Product datasheet

Anti-PP2A alpha + beta antibody [E155] ab32104

Recombinant RabMAb

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Overview

Product name	Anti-PP2A alpha + beta antibody [E155]
Description	Rabbit monoclonal [E155] to PP2A alpha + beta
Host species	Rabbit
Specificity	The immunogen sequence for ab32104 has 100% homology with the PP2A-beta protein.
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, Flow Cyt (Intra), Dot blot
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat, Zebrafish
Immunogen	Synthetic peptide within Human PP2A alpha + beta aa 250-350. The exact sequence is proprietary.
Positive control	IHC-P: Human pancreas tissue. WB: lysate of A431 cells (serum starved overnight) treated with EGF (100ng/ml) for 5-10 minutes at 37C (BD Biosciences).
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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	E155
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab32104 in the following tested applications.

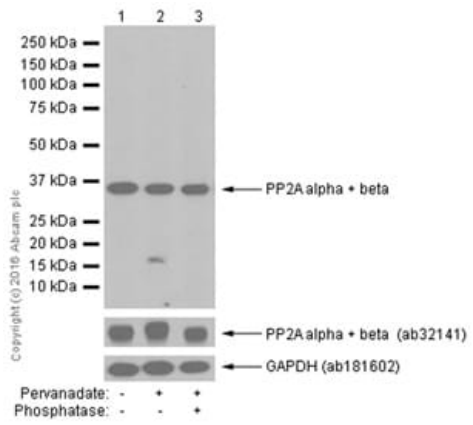
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★ (1)	1/5000. Predicted molecular weight: 35 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF	★★★★★ (1)	Use a concentration of 5 µg/ml.
Flow Cyt (Intra)		Use at an assay dependent concentration. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
Dot blot		Use at an assay dependent concentration.

Target

Function	PP2A can modulate the activity of phosphorylase B kinase casein kinase 2, mitogen-stimulated S6 kinase, and MAP-2 kinase. Cooperates with SGOL2 to protect centromeric cohesin from separase-mediated cleavage in oocytes specifically during meiosis I (By similarity). Can dephosphorylate SV40 large T antigen and p53/TP53. Dephosphorylates SV40 large T antigen, preferentially on serine residues 120, 123, 677, and perhaps 679. The C subunit was most active, followed by the AC form, which was more active than the ABC form, and activity of all three forms was strongly stimulated by manganese, and to a lesser extent by magnesium. Dephosphorylation by the AC form, but not C or ABC form is inhibited by small T antigen. Activates RAF1 by dephosphorylating it at 'Ser-259'.
Sequence similarities	Belongs to the PPP phosphatase family. PP-1 subfamily.
Post-translational modifications	Reversibly methyl esterified on Leu-309. Carboxyl methylation may play a role in holoenzyme assembly, enhancing the affinity of the PP2A core enzyme for some, but not all, regulatory subunits. It varies during the cell cycle. Phosphorylation of either threonine (by autophosphorylation-activated protein kinase) or tyrosine results in inactivation of the phosphatase. Auto-dephosphorylation has been suggested as a mechanism for reactivation.
Cellular localization	Cytoplasm. Nucleus. Chromosome > centromere. Cytoplasm > cytoskeleton > spindle pole. In prometaphase cells, but not in anaphase cells, localizes at centromeres. During mitosis, also found at spindle poles. Centromeric localization requires the presence of SGOL2.

Images



Western blot - Anti-PP2A alpha + beta antibody [E155] (ab32104)

All lanes : Anti-PP2A alpha + beta antibody [E155] (ab32104) at 1/5000 dilution

Lane 1 : Untreated HeLa (human cervix adenocarcinoma) whole cell lysates
Lane 2 : HeLa (human cervix adenocarcinoma) whole cell lysates,cells were treated with Pervanadate for 30 minutes at the final concentration of 1mg/ml
Lane 3 : HeLa (human cervix adenocarcinoma) whole cell lysates,cells were treated with Pervanadate for 30 minutes at the final concentration of 1mg/ml Then the membrane was incubated with Alkaline phosphatase.

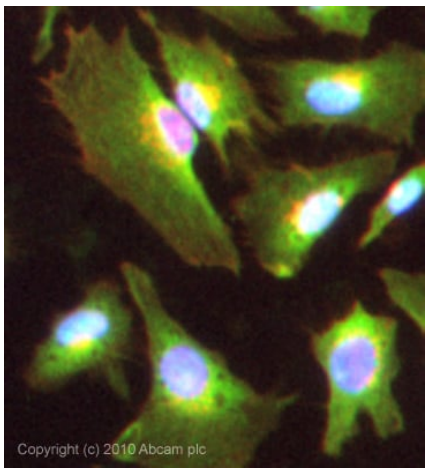
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 35 kDa

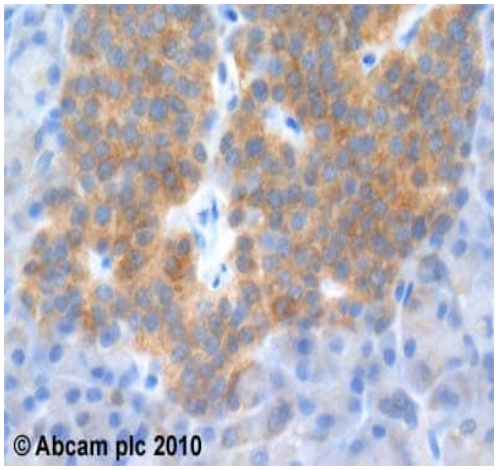
Blocking and Diluting buffer and concentration:5% NFDM/TBST

Exposure time:3 minutes



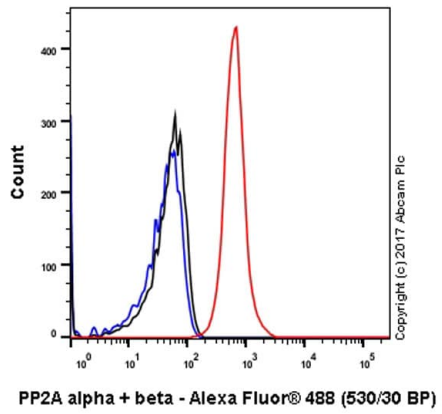
Immunocytochemistry/ Immunofluorescence - Anti-PP2A alpha + beta antibody [E155] (ab32104)

ICC/IF image of ab32104 stained HeLa cells. The cells were 4% formaldehyde 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 (ab32104, 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.



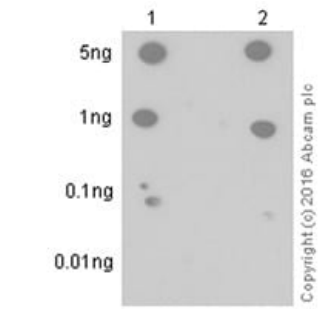
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PP2A alpha + beta antibody [E155] (ab32104)

ab32104 (2µg/ml) staining PP2A alpha in human pancreas using an automated system (DAKO Autostainer Plus). Using this protocol there is strong cytoplasmic staining of the islet of Langerhans. Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer citrate pH6.1 in a DAKO PT link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.



Flow Cytometry (Intracellular) - Anti-PP2A alpha + beta antibody [E155] (ab32104)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling PP2A alpha + beta with purified ab32104 at 1/20 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 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.



Dot Blot - Anti-PP2A alpha + beta antibody [E155] (ab32104)

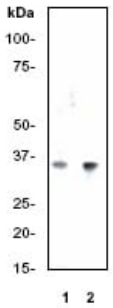
Dot blot was performed using ab32104 at 1/1000 dilution.Goat Anti-Rabbit IgG,(H+L),HRP conjugated (ab97051) was used as secondary antibody at 1/100000 dilution

Lane 1:PP2A alpha + beta phospho peptide

Lane 2:PP2A alpha + beta non-phospho peptide

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

Exposure time:10 seconds



Western blot - Anti-PP2A alpha + beta antibody [E155] (ab32104)

All lanes : Anti-PP2A alpha + beta antibody [E155] (ab32104) at 1/5000 dilution

Lane 1 : untreated A431 cell lysate

Lane 2 : EGF treated A431 cell lysate (the specific EGF concentration and incubation time is confidential information)

Predicted band size: 35 kDa

Observed band size: 35 kDa

Anti-PP2A alpha + beta antibody [E155] (ab32104)

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