abcam

Product datasheet

Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] ab40776

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y]
Description	Rabbit monoclonal [EP383Y] to PI 3 Kinase catalytic subunit alpha/PIK3CA
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IP, Flow Cyt (Intra) **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human PI 3 Kinase catalytic subunit alpha/PIK3CA aa 1000 to the C-terminus (C terminal). The exact sequence is proprietary. Database link: **P42336**
Positive control	WB: Jurkat, MCF-7, Raw264.7 and NIH/3T3 cell lysates. ICC/IF: HeLa and Jurkat cells. IP: Jurkat whole cell lysate (**ab7899**).
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: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EP383Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab40776 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		1/1000 - 1/10000. Detects a band of approximately 110 kDa (predicted molecular weight: 110 kDa).
ICC/IF		1/100 - 1/250.
IP		1/20 - 1/30.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Application notes Is unsuitable for IHC-P.

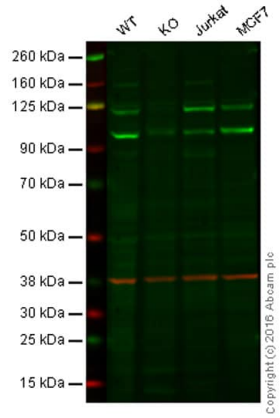
Target

Function Phosphorylates PtdIns, PtdIns4P and PtdIns(4,5)P2 with a preference for PtdIns(4,5)P2.

Involvement in disease Defects in PIK3CA are associated with colorectal cancer (CRC) [MIM:114500].
Defects in PIK3CA are a cause of susceptibility to breast cancer (BC) [MIM:114480]. A common malignancy originating from breast epithelial tissue. Breast neoplasms can be distinguished by their histologic pattern. Invasive ductal carcinoma is by far the most common type. Breast cancer is etiologically and genetically heterogeneous. Important genetic factors have been indicated by familial occurrence and bilateral involvement. Mutations at more than one locus can be involved in different families or even in the same case.
Defects in PIK3CA are a cause of susceptibility to ovarian cancer (OC) [MIM:167000]. Ovarian cancer common malignancy originating from ovarian tissue. Although many histologic types of ovarian neoplasms have been described, epithelial ovarian carcinoma is the most common form. Ovarian cancers are often asymptomatic and the recognized signs and symptoms, even of late-stage disease, are vague. Consequently, most patients are diagnosed with advanced disease.
Defects in PIK3CA may underlie hepatocellular carcinoma (HCC) [MIM:114550].
Defects in PIK3CA are a cause of keratosis seborrheic (KERSEB) [MIM:182000]. A common benign skin tumor. Seborrheic keratoses usually begin with the appearance of one or more sharply defined, light brown, flat macules. The lesions may be sparse or numerous. As they initially grow, they develop a velvety to finely verrucous surface, followed by an uneven warty surface with multiple plugged follicles and a dull or lackluster appearance.

Sequence similarities Belongs to the PI3/PI4-kinase family.
Contains 1 C2 domain.
Contains 1 PI3K/PI4K domain.

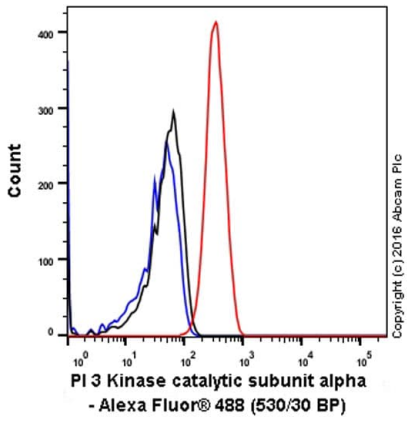
Images



Western blot - Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] (ab40776)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: PI 3 Kinase catalytic subunit alpha/PIK3CA knockout HAP1 cell lysate (20 µg)
Lane 3: Jurkat cell lysate (20 µg)
Lane 4: MCF7 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab40776 observed at 120 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab40776 was shown to recognize PI 3 Kinase catalytic subunit alpha/PIK3CA when PI 3 Kinase catalytic subunit alpha/PIK3CA knockout samples were used, along with additional cross-reactive bands. Wild-type and PI 3 Kinase catalytic subunit alpha/PIK3CA knockout samples were subjected to SDS-PAGE. ab40776 and **ab8245** (loading control to GAPDH) were diluted to 1/1000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

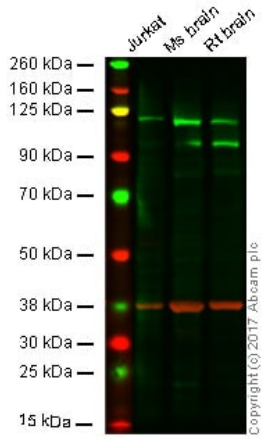


Flow Cytometry (Intracellular) - Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] (ab40776)

ab40776 staining PI 3 Kinase catalytic subunit alpha/PIK3CA in the human cell line Jurkat (human acute T cell leukemia) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde, permiabilised with 90% methanol and the sample was incubated with the primary antibody at a dilution of 1/40. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.

Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)



Western blot - Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] (ab40776)

All lanes : Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] (ab40776) at 1/1000 dilution

Lane 1 : Jurkat Whole Cell Lysate
Lane 2 : Mouse Brain Tissue Lysate
Lane 3 : Rat Brain Tissue Lysate

Lysates/proteins at 20 µg per lane.

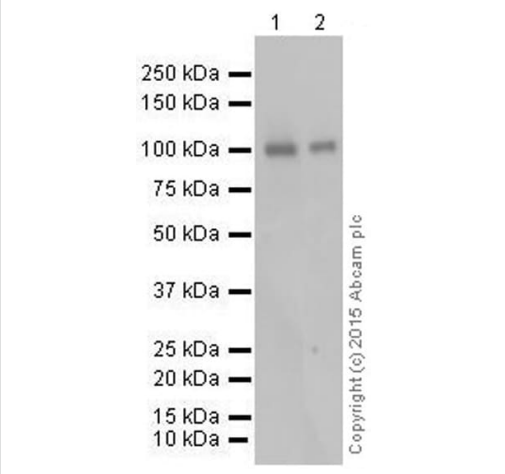
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 110 kDa
Observed band size: 120 kDa

Lanes 1 - 3: Merged signal (red and green). Green - ab40776 observed at 120 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab40776 and **ab8245** (loading control) overnight at 4°C. Antibody binding was detected using Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at a 1:10000 dilution for 1hr at room temperature and then imaged.



Western blot - Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] (ab40776)

All lanes : Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] (ab40776) at 1/5000 dilution (purified)

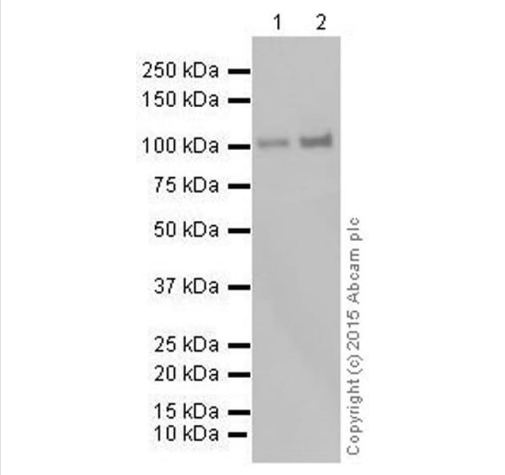
Lane 1 : Jurkat whole cell lysate
Lane 2 : MCF-7 whole cell lysate

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: 110 kDa
Observed band size: 110 kDa

Blocking and dilution buffer: 5% NFDM/TBST



Western blot - Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] (ab40776)

All lanes : Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] (ab40776) at 1/5000 dilution (purified)

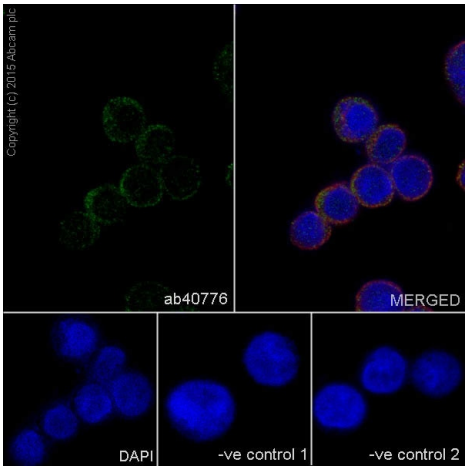
Lane 1 : Raw264.7 whole cell lysate
Lane 2 : NIH/3T3 whole cell lysate

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: 110 kDa
Observed band size: 110 kDa

Blocking and dilution buffer: 5% NFDM/TBST

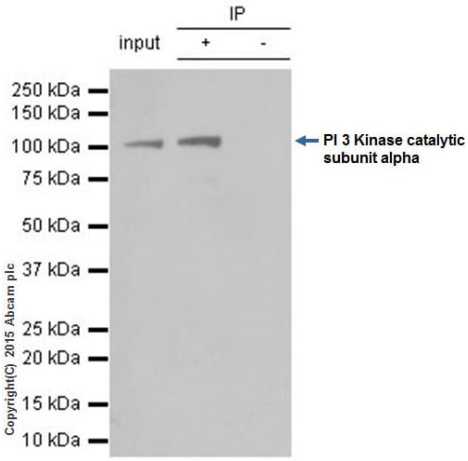


Immunocytochemistry/ Immunofluorescence - Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] (ab40776)

Immunocytochemistry/Immunofluorescence analysis of Jurkat cells labelling PI 3 Kinase catalytic subunit alpha/PIK3CA with purified ab40776 at a dilution of 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).



Immunoprecipitation - Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] (ab40776)

ab40776 (purified) at a dilution of 1/20 immunoprecipitating PI 3 Kinase catalytic subunit alpha/PIK3CA in Jurkat whole cell lysate.

Lane 1 (input): Jurkat whole cell lysate (10µg)

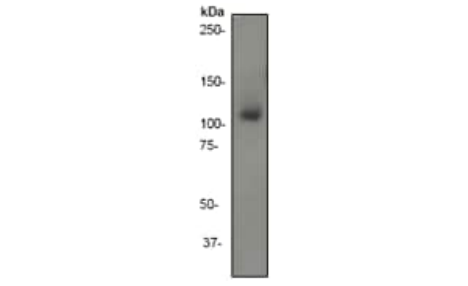
Lane 2 (+): ab40776 + Jurkat whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab40776 in Jurkat whole cell lysate.

For western blotting, **ab131366** VeriBlot for IP (HRP) was used for detection (1/1000).

Blocking buffer and concentration: 5% NFDM/TBST.

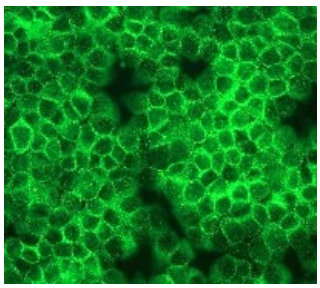
Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] (ab40776)

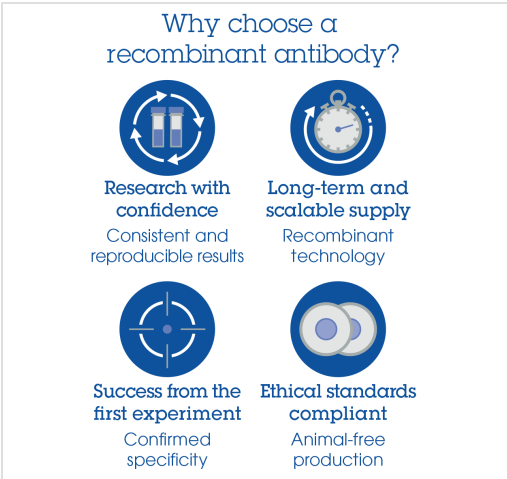
Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] (ab40776) at 1/5000 dilution (unpurified) + Jurkat cell lysate at 10 µg

Predicted band size: 110 kDa
Observed band size: 110 kDa



Immunocytochemistry/ Immunofluorescence - Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] (ab40776)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling PI 3 Kinase catalytic subunit alpha/PIK3CA with unpurified ab40776 at a dilution of 1/100.



Anti-PI 3 Kinase catalytic subunit alpha/PIK3CA antibody [EP383Y] (ab40776)

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