

## Product datasheet

# Anti-PTEN (phospho T366) antibody [EP229] ab109454

Recombinant RabMAb

[12 References](#) [6 Images](#)

### Overview

<b>Product name</b>	Anti-PTEN (phospho T366) antibody [EP229]
<b>Description</b>	Rabbit monoclonal [EP229] to PTEN (phospho T366)
<b>Host species</b>	Rabbit
<b>Specificity</b>	The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
<b>Tested applications</b>	<b>Suitable for:</b> WB, IHC-P, Dot blot <b>Unsuitable for:</b> Flow Cyt
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human
<b>Immunogen</b>	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	WB: HeLa (Human cervix adenocarcinoma epithelial cell), NIH/3T3 (Mouse embryonic fibroblast), and C6 (Rat glial tumor glial cell) whole cell lysate untreated, treated with 100ng/ml Calyculin A for 30 minutes, and ted with 100ng/ml Calyculin A for 30 minutes whole cell lysate then the membrane was incubated with Alkaline phosphatase. IHC-P: Human breast ductal carcinoma tissue and prostate cancer sections.
<b>General notes</b>	<p>This product has switched from a hybridoma to recombinant production method on 9th June 2023.</p> <p>PTEN is a protein implicated in several disease, including certain cancers and neurological diseases. PTEN is expressed ubiquitously throughout the body and acts as a phosphatase to dephosphorylate phosphatidylinositol (3,4,5)-trisphosphate. This is important in the inhibition of the Akt signalling pathway, which plays an important role in regulating cellular behaviours such as cell growth, survival, and migration.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

## Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
<b>Storage buffer</b>	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EP229
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab109454 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
<b>WB</b>		1/10000. Detects a band of approximately 54 kDa (predicted molecular weight: 47 kDa).
<b>IHC-P</b>		1/2000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
<b>Dot blot</b>		1/10000.

**Application notes** Is unsuitable for Flow Cyt.

## Target

**Function** Tumor suppressor. Acts as a dual-specificity protein phosphatase, dephosphorylating tyrosine-, serine- and threonine-phosphorylated proteins. Also acts as a lipid phosphatase, removing the phosphate in the D3 position of the inositol ring from phosphatidylinositol 3,4,5-trisphosphate, phosphatidylinositol 3,4-diphosphate, phosphatidylinositol 3-phosphate and inositol 1,3,4,5-tetrakisphosphate with order of substrate preference in vitro  $\text{PtdIns}(3,4,5)\text{P}_3 > \text{PtdIns}(3,4)\text{P}_2 > \text{PtdIns}3\text{P} > \text{Ins}(1,3,4,5)\text{P}_4$ . The lipid phosphatase activity is critical for its tumor suppressor function. Antagonizes the PI3K-AKT/PKB signaling pathway by dephosphorylating phosphoinositides and thereby modulating cell cycle progression and cell survival. The unphosphorylated form cooperates with AIP1 to suppress AKT1 activation. Dephosphorylates tyrosine-phosphorylated focal adhesion kinase and inhibits cell migration and integrin-mediated cell spreading and focal adhesion formation. Plays a role as a key modulator of the AKT-mTOR signaling pathway controlling the tempo of the process of newborn neurons integration during adult neurogenesis, including correct neuron positioning, dendritic development and synapse formation. May be a negative regulator of insulin signaling and glucose metabolism in adipose tissue. The nuclear monoubiquitinated form possesses greater apoptotic potential, whereas the

cytoplasmic nonubiquitinated form induces less tumor suppressive ability. In motile cells, suppresses the formation of lateral pseudopods and thereby promotes cell polarization and directed movement.

Isoform alpha: Functional kinase, like isoform 1 it antagonizes the PI3K-AKT/PKB signaling pathway. Plays a role in mitochondrial energetic metabolism by promoting COX activity and ATP production, via collaboration with isoform 1 in increasing protein levels of PINK1.

#### **Tissue specificity**

Expressed at a relatively high level in all adult tissues, including heart, brain, placenta, lung, liver, muscle, kidney and pancreas.

#### **Involvement in disease**

Cowden syndrome 1

Lhermitte-Duclos disease

Bannayan-Riley-Ruvalcaba syndrome

Squamous cell carcinoma of the head and neck

Endometrial cancer

PTEN mutations are found in a subset of patients with Proteus syndrome, a genetically heterogeneous condition. The molecular diagnosis of PTEN mutation positive cases classifies Proteus syndrome patients as part of the PTEN hamartoma syndrome spectrum. As such, patients surviving the early years of Proteus syndrome are likely at a greater risk of developing malignancies.

Glioma 2

VACTERL association with hydrocephalus

Prostate cancer

Macrocephaly/autism syndrome

A microdeletion of chromosome 10q23 involving BMPR1A and PTEN is a cause of chromosome 10q23 deletion syndrome, which shows overlapping features of the following three disorders: Bannayan-Zonana syndrome, Cowden disease and juvenile polyposis syndrome.

#### **Sequence similarities**

Contains 1 C2 tensin-type domain.

Contains 1 phosphatase tensin-type domain.

#### **Domain**

The C2 domain binds phospholipid membranes in vitro in a Ca(2+)-independent manner; this binding is important for its tumor suppressor function.

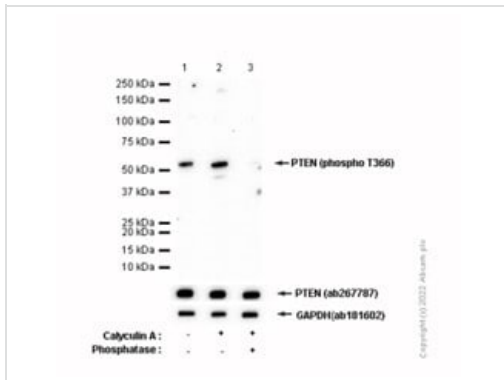
#### **Post-translational modifications**

Constitutively phosphorylated by CK2 under normal conditions. Phosphorylated in vitro by MAST1, MAST2, MAST3 and STK11. Phosphorylation results in an inhibited activity towards PIP3. Phosphorylation can both inhibit or promote PDZ-binding. Phosphorylation at Tyr-336 by FRK/PTK5 protects this protein from ubiquitin-mediated degradation probably by inhibiting its binding to NEDD4. Phosphorylation by ROCK1 is essential for its stability and activity. Phosphorylation by PLK3 promotes its stability and prevents its degradation by the proteasome. Monoubiquitinated; monoubiquitination is increased in presence of retinoic acid. Deubiquitinated by USP7; leading to its nuclear exclusion. Monoubiquitination of one of either Lys-13 and Lys-289 amino acid is sufficient to modulate PTEN compartmentalization. Ubiquitinated by XIAP/BIRC4.

#### **Cellular localization**

Secreted. May be secreted via a classical signal peptide and reenter into cells with the help of a poly-Arg motif and Cytoplasm. Nucleus. Nucleus, PML body. Monoubiquitinated form is nuclear. Nonubiquitinated form is cytoplasmic. Colocalized with PML and USP7 in PML nuclear bodies. XIAP/BIRC4 promotes its nuclear localization.

#### **Images**



Western blot - Anti-PTEN (phospho T366) antibody [EP229] (ab109454)

**All lanes** : Anti-PTEN (phospho T366) antibody [EP229] - BSA and Azide free ([ab208104](#)) at 1/100000 dilution

**Lane 1** : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

**Lane 2** : HeLa (Human cervix adenocarcinoma epithelial cell) treated with 100ng/ml Calyculin A for 30 minutes whole cell lysate

**Lane 3** : HeLa (Human cervix adenocarcinoma epithelial cell) treated with 100ng/ml Calyculin A for 30 minutes whole cell lysate, then the membrane was incubated with Alkaline phosphatase

Lysates/proteins at 15 µg per lane.

### Secondary

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

Developed using the ECL technique.

Performed under reducing conditions.

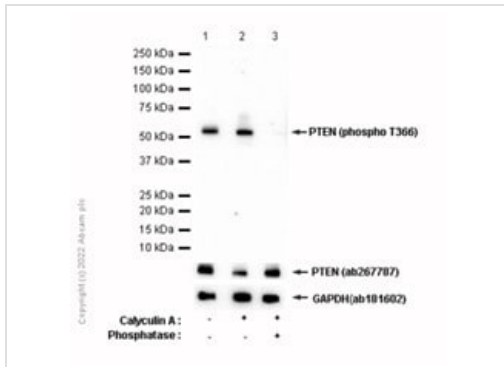
**Predicted band size:** 47 kDa

**Exposure time:** 80 seconds

This data was developed using the same antibody clone in a different buffer formulation - BSA and Azide free ([ab208104](#)).

Recommended concentration for ab109454: 1/10000 dilution (0.1ug/ml).

Blocking and diluting buffer and concentration: 5% NFDm/TBST, Primary and secondary antibodies were incubated for 1h at room temperature.



Western blot - Anti-PTEN (phospho T366) antibody [EP229] (ab109454)

**All lanes** : Anti-PTEN (phospho T366) antibody [EP229] - BSA and Azide free ([ab208104](#)) at 1/100000 dilution

**Lane 1** : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

**Lane 2** : NIH/3T3 (Mouse embryonic fibroblast) treated with 100 ng/ml Calyculin A for 30 minutes whole cell lysate

**Lane 3** : NIH/3T3 (Mouse embryonic fibroblast) treated with 100 ng/ml Calyculin A for 30 minutes whole cell lysate, then the membrane was incubated with Alkaline phosphatase.

Lysates/proteins at 15 µg per lane.

## Secondary

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

Developed using the ECL technique.

Performed under reducing conditions.

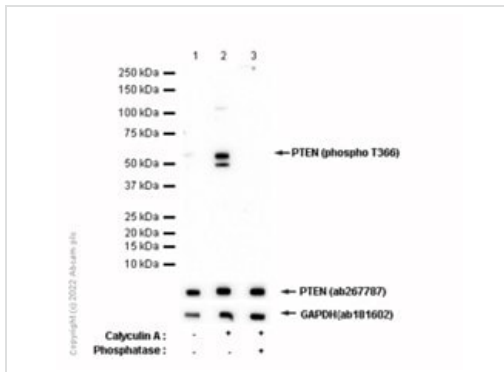
**Predicted band size:** 47 kDa

**Exposure time:** 40 seconds

This data was developed using the same antibody clone in a different buffer formulation - BSA and Azide free ([ab208104](#)).

Recommended concentration for ab109454: 1/10000 dilution (0.1 µg/ml).

Blocking and diluting buffer and concentration: 5% NFDm/TBST, Primary and secondary antibodies were incubated for 1h at room temperature.



Western blot - Anti-PTEN (phospho T366) antibody [EP229] (ab109454)

**All lanes** : Anti-PTEN (phospho T366) antibody [EP229] - BSA and Azide free ([ab208104](#)) at 1/100000 dilution

**Lane 1** : C6 (Rat glial tumor glial cell) whole cell lysate

**Lane 2** : C6 (Rat glial tumor glial cell) treated with 100 ng/ml Calyculin A for 30 minutes whole cell lysate

**Lane 3** : C6 (Rat glial tumor glial cell) treated with 100 ng/ml Calyculin A for 30 minutes whole cell lysate, then the membrane was incubated with Alkaline phosphatase

Lysates/proteins at 15 µg per lane.

### Secondary

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

Developed using the ECL technique.

Performed under reducing conditions.

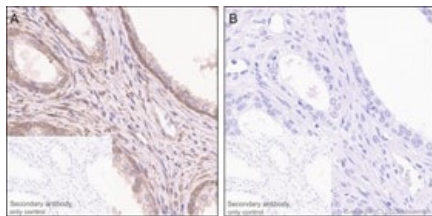
**Predicted band size:** 47 kDa

**Exposure time:** 20 seconds

This data was developed using the same antibody clone in a different buffer formulation - BSA and Azide free ([ab208104](#)).

Recommended concentration for ab109454: 1/10000 dilution (0.1 µg/ml).

Blocking and diluting buffer and concentration: 5% NFDm/TBST, Primary and secondary antibodies were incubated for 1h at room temperature.



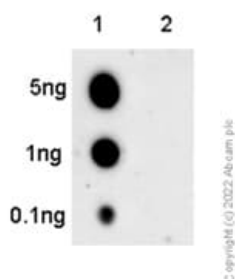
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-PTEN (phospho T366) antibody [EP229] (ab109454)

This data was developed using the same antibody clone in a different buffer formulation - BSA and Azide free ([ab208104](#)).

Recommended concentration for ab109454: 1/200 dilution.

The human prostate cancer sections were performed by Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins, then treated with or without alkaline phosphatase. After alkaline phosphatase treatment, the sections were labelled with [ab208104](#) at 1/2000 dilution for 30 mins at room temperature. The Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)) were used as secondary antibody. The sections Counterstained with hematoxylin. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Human prostate cancer without alkaline phosphatase treatment (image A), no signal was detected when treated with alkaline phosphatase (image B).



Dot Blot - Anti-PTEN (phospho T366) antibody [EP229] (ab109454)

This data was developed using the same antibody clone in a different buffer formulation - BSA and Azide free ([ab208104](#)).

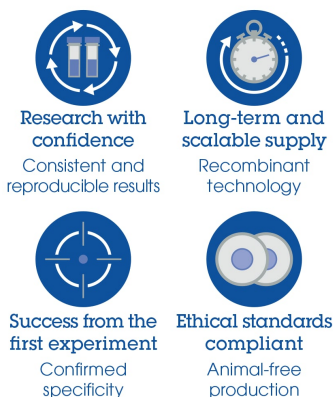
Recommended concentration for ab109454: 1/1000 dilution.

Dot blot analysis of PTEN (pT366) peptide (Lane 1), and PTEN non-phospho peptide (Lane 2) incubated with [ab208104](#) at a dilution of 1/10000.

[ab97051](#) (Peroxidase conjugated goat anti-rabbit IgG (H+L)) was used as the secondary antibody at a dilution of 1/100,000.

Exposure time: 180s

### Why choose a recombinant antibody?



Anti-PTEN (phospho T366) antibody [EP229] (ab109454)

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

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