Product datasheet

Anti-PUMA antibody ab9643

Overview

Product name  Anti-PUMA antibody
Description  Rabbit polyclonal to PUMA
Host species  Rabbit
Specificity  At least 2 isoforms are known to exist; this antibody will detect both isoforms.
Tested applications  Suitable for: WB, IHC-P, ICC/IF
Species reactivity  Reacts with: Mouse, Rat, Human
Immunogen  Synthetic peptide: PLPRGHRAPEMEPN, corresponding to C terminal amino acids 180-193 of Human PUMA alpha. (Peptide available as ab9644.)

Positive control  WB: K562, HEK293 and NIH3T3 ICC/IF: K562 cells.

General notes

Apoptosis is related to many diseases and development. The p53 tumor-suppressor protein induces apoptosis through transcriptional activation of several genes. A novel p53 inducible pro-apoptotic gene was identified recently and designated PUMA (for p53 upregulated modulator of apoptosis) and bbc3 (for Bcl-2 binding component 3) in human and mouse (1-3). PUMA/bbc3 is one of the pro-apoptotic Bcl-2 family members including Bax and Noxa, which are also transcriptional targets of p53. The PUMA gene encodes two BH3 domain-containing proteins termed PUMA-a and PUMA-b (1). PUMA proteins bind Bcl-2, localize to the mitochondria, and induce cytochrome c release and apoptosis in response to p53. PUMA may be a direct mediator of p53-induced apoptosis.

Properties

Form  Liquid
Storage instructions  Shipped at 4°C. Store at +4°C.
Storage buffer  Preservative: 0.02% Sodium azide
                Constituent: PBS
Purity  Immunogen affinity purified
Primary antibody notes  Apoptosis is related to many diseases and development. The p53 tumor-suppressor protein
induces apoptosis through transcriptional activation of several genes. A novel p53 inducible pro-apoptotic gene was identified recently and designated PUMA (for p53 upregulated modulator of apoptosis) and bbc3 (for Bcl-2 binding component 3) in human and mouse (1-3). PUMA/bbc3 is one of the pro-apoptotic Bcl-2 family members including Bax and Noxa, which are also transcriptional targets of p53. The PUMA gene encodes two BH3 domain-containing proteins termed PUMA-a and PUMA-b (1). PUMA proteins bind Bcl-2, localize to the mitochondria, and induce cytochrome c release and apoptosis in response to p53. PUMA may be a direct mediator of p53-induced apoptosis.

Clonality  Polyclonal
Isotype  IgG

Applications

Our Abpromise guarantee covers the use of ab9643 in the following tested applications.

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>🌟🌟🌟🌟🌟</td>
<td>Use a concentration of 1 - 2 µg/ml. Detects a band of approximately 23 kDa. Can be blocked with Human PUMA peptide (ab9644). Use at a concentration of 1 - 2 µg/ml. Detects a band of approximately 23 kDa. Can be blocked with PUMA peptide (180/193) (ab9644). A lower band at approximately 16 kDa was detected in MOLT4 and U937 cells, which may represent the PUMA-beta form.</td>
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<tr>
<td>IHC-P</td>
<td>🌟🌟🌟🌟🌟</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>🌟🌟🌟🌟🌟</td>
<td>Use a concentration of 1 µg/ml.</td>
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</tbody>
</table>

Target

Function  Essential mediator of p53-dependent and p53-independent apoptosis.
Tissue specificity  Ubiquitously expressed.
Sequence similarities  Belongs to the Bcl-2 family.
Cellular localization  Mitochondrion. Localized to the mitochondria in order to induce cytochrome c release.

Images
**Western blot** - Anti-PUMA antibody (ab9643)

**All lanes**: Anti-PUMA antibody (ab9643) at 2 µg/ml

**Lane 1**: HEK293 cells were transfected with control siRNAs with control siRNAs

**Lane 2**: HEK293 cells were transfected with PUMA siRNAs with PUMA siRNA

Lysates/proteins at 15 µg per lane.

**Secondary**

**All lanes**: Goat anti-rabbit IgG HRP conjugate at 1/10000 dilution

Beta-actin (1 µg/mL) and GAPDH (0.02 µg/mL). Incubation time: 1 hour at Room Temperature in 5% NFDM/TBST.

Immunofluorescent analysis of 4% paraformaldehyde fixed K562 cells labeling PUMA with ab9643 at 2 µg/mL, followed by goat anti-rabbit IgG secondary antibody at 1/500 dilution (red). Image showing cytosol staining on K562 cells.
ab9643 staining rat gonad tissue sections by Immunohistochemistry (IHC-P - Formalin/PFA-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and blocked with 1.5% milk for 45 minutes at 25°C. Protease/PBS (100mg/200ml) was used for antigen retrieval, \( \text{H}_2\text{O}_2 \)/methanol solution (0.3%) was used to destroy endogenous peroxidase activity. Samples were incubated with the primary antibody (1/500) for 12 hours at 4°C. A Biotin-conjugated goat anti-rabbit IgG polyclonal (1/200) was used as the secondary antibody.

ICC/IF image of ab9643 stained HeLa 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 (ab9643, 1µg/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.
Immunocytochemical analysis of K562 cells labeling PUMA with ab9643 at 1 µg/mL. Cells were fixed with formaldehyde and blocked with 10% serum for 1 hour at room temperature. Antigen retrieval was by heat mediation with a citrate buffer (pH 6.0). Samples were incubated with primary antibody overnight at 4°C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.

**Western blot - Anti-PUMA antibody (ab9643)**

*All lanes*: Anti-PUMA antibody (ab9643) at 2 µg/ml

*Lane 1*: K562 cell lysate

*Lane 2*: NIH3T3 cell lysate

Lysates/proteins at 15 µg per lane.

**Secondary**

*All lanes*: Goat anti-rabbit IgG HRP conjugate at 1/10000 dilution

Anti-PUMA antibody (ab9643) at 1/1000 dilution + HeLa whole cell lysate

**Secondary**

Alexa Fluor 680-conjugated goat anti-rabbit IgG polyclonal at 1/1 dilution

**Observed band size**: 20 kDa

*why is the actual band size different from the predicted?*

**Additional bands at**: 26 kDa (possible non-specific binding), 42 kDa (possible non-specific binding), 50 kDa (possible non-specific binding)

**Exposure time**: 5 seconds

Blocked with 5% milk for 1 hour.

Incubated with the primary antibody for 18 hours.
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