Human/Mouse/Rat PTEN Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF847

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat PTEN in Western blots.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	Human PTEN synthetic peptide Ser385-Val403 Accession # P60484
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

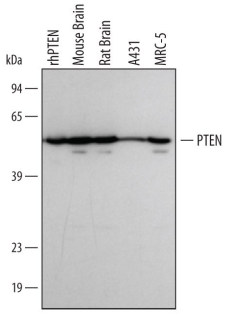
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.1 µg/mL	See Below
Immunohistochemistry	3-15 µg/mL	See Below
Intracellular Staining by Flow Cytometry	0.25 µg/10^6 cells	See Below
Simple Western	1 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Knockout Validated	PTEN is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in PTEN knockout HeLa cell line.	

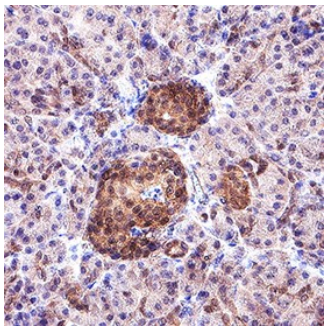
DATA

Western Blot



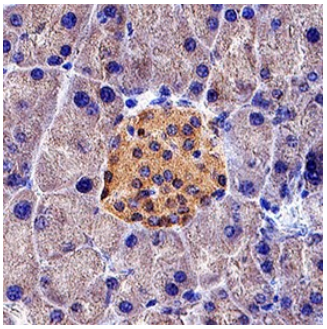
Detection of Human/Mouse/Rat PTEN by Western Blot. Western blot shows lysates of mouse and rat brain tissue, A431 human epithelial carcinoma cell line, and MRC-5 human embryonic lung fibroblast cell line. PVDF membrane was probed with 0.1 µg/mL Rabbit Anti-Human/Mouse/Rat PTEN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF847) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # Catalog # HAF008). For additional reference, recombinant human PTEN (5 ng) was included. A specific band for PTEN was detected at approximately 54 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 4.

Immunohistochemistry



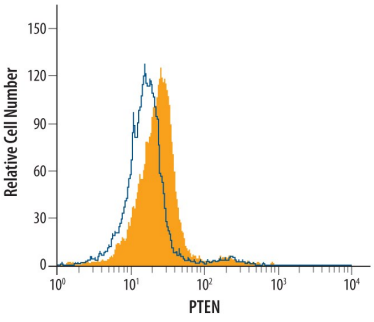
PTEN in Human Pancreas. PTEN was detected in immersion fixed paraffin-embedded sections of human pancreas using Rabbit Anti-Human/Mouse/Rat PTEN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF847) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm in islet cells. Staining was performed using our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Immunohistochemistry



PTEN in Mouse Pancreas. PTEN was detected in immersion fixed paraffin-embedded sections of mouse pancreas using Rabbit Anti-Human/Mouse/Rat PTEN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF847) at 3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm in islet cells. Staining was performed using our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Intracellular Staining by Flow Cytometry



Detection of PTEN in Human PBMC lymphocytes by Flow Cytometry. Human peripheral blood lymphocytes were stained with Rabbit Anti-Human/Mouse/Rat PTEN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF847, filled histogram) or control antibody (Catalog # Catalog # AB-105-C, open histogram), followed by Phycoerythrin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # Catalog # F0110). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.

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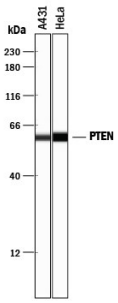
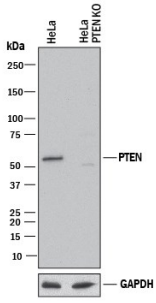
Simple Western



Detection of Human PTEN by Simple Western™. Simple Western lane view shows lysates of A431 human epithelial carcinoma cell line and HeLa human cervical epithelial carcinoma cell line, loaded at 0.2 mg/mL. A specific band was detected for PTEN at approximately 60 kDa (as indicated) using 1 µg/mL of Rabbit Anti-Human/Mouse/Rat PTEN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF847). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Knockout Validated



Western Blot Shows Human PTEN Specificity by Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and PTEN knockout HeLa cell line (KO). PVDF membrane was probed with 0.1 µg/mL of Rabbit Anti-Human/Mouse/Rat PTEN Antigen Affinity-purified Polyclonal Antibody (Catalog # AF847) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # Catalog # HAF008). A specific band was detected for PTEN at approximately 55 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

The tumor suppressor gene PTEN (phosphatase and tensin homolog deleted on chromosome 10), also known as MMAC1 (mutated in multiple advanced cancers 1), encodes a phosphatase that contains the catalytic signature motif (HCXXGXXRS/T) found in all members of the protein tyrosine phosphatase family. *In vitro*, the recombinant PTEN has both lipid phosphatase and protein phosphatase activities (1, 2). Interestingly, accumulating evidence has shown that the tumor suppressor activity of PTEN relies on its ability to dephosphorylate phosphatidylinositol (3, 4, 5)-triphosphate specifically at position 3 of the inositol ring (3). This activity reduces the levels of phosphatidylinositol (3, 4, 5)-triphosphate which is specifically produced from phosphatidylinositol (4, 5)-diphosphate by PI 3-kinase upon activation by a variety of stimuli. Therefore, PTEN antagonizes PI 3-kinase-induced downstream signaling events and cellular processes including cell growth, apoptosis and cell motility. *In vivo*, the importance of PTEN catalytic activity in its tumor suppressor functions is underscored by the fact that the majority of PTEN missense mutations detected in tumor specimens target the phosphatase domain and cause a loss in PTEN phosphatase activity (4).

References:

1. Maehama, T. and J. Dixon (1998) J. Biol. Chem. **273**:13375.
2. Das, S. *et al.* (2003) Proc. Natl. Acad. Sci. USA **100**:7491.
3. Myers, M. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:13513.
4. Waite, K. and C. Eng (2002) Am. J. Hum. Genet. **70**:829.

PRODUCT SPECIFIC NOTICES

This product is covered by the following U.S. patent: USSN # 10/299,003.

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