Rabbit (polyclonal) Anti-PTEN [pSpTpS$^{380/382/385}$] Phosphospecific Antibody, Unconjugated

Catalog no. 441066G

(See product label for lot information)

Clone/PAD: pAb
Isotype: Rb IgG
Gene ID: PTEN
Qty: 10 mini-blot size
Volume: 100 µL

Formulation
Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol with 1.0 mg/mL BSA (IgG, protease free) as a carrier.

Validation
See www.invitrogen.com/antibodies for protocols
Validated for use in Western Blot.

Reactivity
Human and mouse PTEN. Rat PTEN (100% homologous) has not been tested, but is expected to react.

Immunogen
Synthetic phosphopeptide from human PTEN containing serine 380, threonine 382, and serine 385. The sequence is conserved in mouse and rat.

Sequence Identity
Human and Mouse

Sequence Homology
Rat

Storage
Store at −20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at −20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.

Expiration Date
Expires one year from date of receipt when stored as instructed.

Background
PTEN (Phosphatase and tensin analog) is a ~58-60 kDa lipid phosphatase and tumor suppressor protein that is frequently mutated in tumor cells. It plays a key role in cell migration, survival and apoptosis by negatively regulating phosphoproteins in the Akt/PI3Kinase pathway. The non-catalytic regulatory carboxyl terminus of PTEN contains multiple phosphorylation sites including two CK2 sites (serines 370 and 385), and MAGI-2 sites (threonines 382 and 383). Phosphorylation of serine 370 and 385 primes PTEN for additional phosphorylation of nearby residues while phosphorylation of serine 380 and threonines 382 and 385 plays a critical role in regulating PTEN stability.

Applications
The antibody has been used in Western blotting. Other applications may work but have not been tested.

Application Use
For Western blotting applications, we recommend using the antibody at a 1:1,000 starting dilution. The exact concentration is not determined for each lot, however the typical range is 0.1-1.0 mg/mL. The optimal antibody concentration should be determined empirically for each specific application.

Test Material
3T3-L1 adipocytes or Hek293 cells overexpressing PTEN.

Purification
Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively preadsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated PTEN. The final product is generated by affinity chromatography using a PTEN-derived peptide that is phosphorylated at serine 380, threonine 382, and serine 385.

Preservative:
0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)

This product is for research use only. Not for use in diagnostic procedures.

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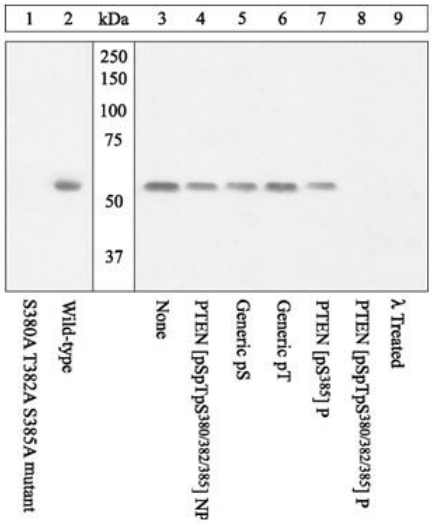

Antibody-Peptide Competition, Mutant Analysis and Phosphatase Stripping

Extracts of Hek293 transiently transfected with a PTEN S380A/T382A/S385A mutant (1) or with wild-type PTEN (2), or extracts of 3T3-L1 cells (3-9) were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF. Membranes were either left untreated (1-8) or treated with lambda (λ) phosphatase (9), blocked with a 3% milk-TBST buffer for one hour at room temperature, then incubated with the PTEN [pSpTpS$^{380/382/385}$] antibody for two hours at room temperature in a 3% milk-TBST buffer, following prior incubation with: no peptide (1-3, 9), the non-phosphopeptide corresponding to the phosphopeptide immunogen (4), a generic phosphoserine-containing peptide (5), a generic phosphothreonine-containing peptide (6), the phosphopeptide corresponding to PTEN [pS^{385}] (7), or the phosphopeptide immunogen (8). After washing, the membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ method.

The data show that only the phosphopeptide corresponding to PTEN [pSpTpS$^{380/382/385}$] blocks the antibody signal, and that the signal is lost with the S380A/T382A/S385A triple mutant (provided by Dr. A. Hall), demonstrating the specificity of the antibody. The data also show that phosphatase stripping eliminates the signal, further verifying that the antibody is phospho-specific.

References

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5. Birle, D., et al. (2002) Negative feedback regulation of the tumor suppressor PTEN by phosphoinositide-induced serine phosphorylation. J. Immunol. 169(1):286-291.
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7. Persad, S., et al. (2001) Tumor suppressor PTEN inhibits nuclear accumulation of beta-catenin and T cell/lymphoid enhancer factor 1-mediated transcriptional activation. J. Cell. Biol. 153(6):1161-1174.
8. Torres, J. and R. Pulido (2001) The tumor suppressor PTEN is phosphorylated by the protein kinase CK2 at its C terminus. Implications for PTEN stability to proteasome-mediated degradation. J. Biol. Chem. 276(2):993-998.