PTEN Phospholipid Phosphatase, active

Product Number: **E-3000**

Sizes: 2.5 µg, 10 µg lyophilized PTEN enzyme

Description: N-terminal GST-tagged, recombinant human PTEN, 76 kD, purified from *E.coli* using glutathione-sepharose column chromatography.

PTEN is a 3' phosphoinositide phosphatase that converts PI(3,4,5)P_3 back to PI(4,5)P_2[1, 2] thus opposing PKB/Akt activation by PI3-K.[3, 4] PTEN is involved in neuronal stem cell proliferation and self-renewal,[5, 6] cardiac myocyte hypertrophy[7] and contractility[8], and a wide range of developmental processes.[9] PTEN, however, is best known for its role as a tumor suppressor.[10] Loss of PTEN activity results in accumulation of PI(3,4,5)P_3, abnormal activation of PKB/Akt, unregulated cell growth[11], suppression of apoptosis,[3, 12] and increased tumorigenesis in a number of human tissues.[13] It has also been proposed that PTEN is a candidate for targeted chemotherapy because certain anti-cancer agents preferentially destroy tumors with PTEN mutations.[14] In addition to this direct role in cancer, PTEN also indirectly regulates cancer-associated pathways including VEGF-mediated angiogenesis among others.[15]

Reconstitution: Reconstitute vial of PTEN with ddH_2O for a 50 µg/mL stock solution prior to use. Flip the vial a few times to mix gently. Spin down and keep on ice. Do not vortex the enzyme.

Storage: Store lyophilized enzyme at -70°C or below. For optimal results, use the entire vial after reconstitution. In our test, reconstituted PTEN retains ~60% activity after overnight storage at 4°C. If the customer chooses to save reconstituted enzyme for longer term, flash freeze PTEN in working aliquots in liquid nitrogen and store aliquots at -70°C or below. PTEN, in solution, may lose more than half of its activity with one freeze/thaw cycle.

Specificity: PTEN selectively removes phosphate from the 3' position of the inositol ring of PtdIns(3,4,5)P_3.

Unit Definition: 1 Unit of PTEN activity is defined as the release of 1 nmol free phosphate per minute at 37°C using PtdIns(3,4,5)P_3 (PIP_3, Product # P-3908) as a substrate in a Malachite Green based phosphatase assay (Product # K-1500).

Assay Conditions: PTEN reaction buffer: TBS (25 mM Tri·HCl, pH 7.4, 140 mM NaCl and 2.7 mM KCl), supplement with 10 mM DTT prior to use.

Recommended assay condition: 30 min to 60 min PTEN reactions at 37°C with 2-4 µg/mL PTEN enzyme and 120 µM PI(3,4,5)P_3 in freshly made PTEN reaction buffer. Avoid vigorously shaking the reactions.

For PTEN inhibitor study, it is recommended to do a PTEN titration for each lot of PTEN received.

QA/Product Testing: See Certificate of Analysis for lot specific information.

Related Products: Malachite Green Assay: K-1500

PTEN ELISA kit:	K-4700
PTEN Substrates:	P-3908, P-3916, P-3916a, and P-3924
Other Lipid Phosphatase:	E-1000 (SHIP2)

References:

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(10) N.R. Leslie and C.P. Downes (2004) PTEN function: how normal cells control it and tumour cells lose it. *Biochem J*, **382**, 1-11.

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PTEN Malachite Green Assay Protocol

Buffer and Reagent Preparation:

- **TBS:** 25 mM Tris-Cl, pH 7.4, 140 mM NaCl, 2.7 mM KCl. Store at room temperature.
- **PTEN Reaction Buffer:** TBS with 10 mM DTT. Make fresh before use and keep on ice. For 4 mL: Add 40 µL of 1 M DTT to 4 mL TBS
- **Phosphate Standards and Malachite Green Solution:** 1 mM phosphate standard and Malachite Green solution are provided in the Malachite Green Assay kit (Product # K-1500). Make standard dilutions in PTEN Reaction Buffer following the kit protocol. Bring Malachite Green solution to room temperature before use.
- **PIP_3 Substrate:** Reconstitute PIP_3 powder (Product # P-3908) in ddH_2O to make a 1 mM stock solution. Use 3 µL (3,000 pmol) for each assay point. Store PIP_3 stock solution at -20°C after use.

PTEN Reaction Setup Table

Sample	PTEN Enzyme	PIP_3, µL	PTEN Reaction Buffer
Buffer Blank control	-	-	25 µL
PTEN (Enzyme-only Control, optional)	50 - 150 ng	-	to final vol. of 25 µL
PIP_3 (Substrate-only Control)	-	3	22 µL
PTEN + PIP_3 (Enzyme Reaction)	50 - 150 ng	3	to final vol. of 25 µL

Assay Setup:

1. Add phosphate standard solutions 25 µL/well in triplicates to a 96-well clear assay plate.
2. Prepare enzyme reactions in triplicate wells according to the table above.
   a. Add PTEN reaction buffer first to corresponding wells.
   b. Reconstitute each vial of PTEN enzyme with ddH2O for a 50 ng/µL PTEN working solution. Flip the vial a few times to mix the enzyme gently. Centrifuge briefly to collect solution at the bottom of the vial and keep on ice. Add 1 µL - 3 µL PTEN working solution (50 ng - 150 ng PTEN) to wells accordingly.
   c. Finally add 3 µL of 1 mM PIP_3 substrate to each substrate-only control and PTEN reaction wells to start reaction. Mix briefly by tapping the plate gently. Seal the plate and incubate at 37°C for 30 to 60 min without shaking.

   Note: *PTEN is very sensitive to temperature and freeze/thaw cycles after reconstitution. For best result, use the entire vial of enzyme in one day. Reconstituted PTEN loses ~40% activity overnight at 4°C.*
3. Add 100 µL/well room temperature Malachite Green solution to each well of 25 µL phosphate standards, controls, and PTEN reactions. Seal plate and cover with aluminum foil to protect from light. Incubate without shaking for 15 to 20 minutes at room temperature to develop green color.
4. Tap plate to mix and read absorbance at 620 nm.
5. Draw standard curve with Abs. 620 nm as Y axis and pmol free phosphate as X axis (We use Polynomial 2nd order non-linear regression correlation).
6. Determine pmol free phosphate from each reaction or control by interpolation from the standard curve.
7. Calculate PIP_3 percentage conversion as follows:

$$PIP_3\ \%\ \text{conversion} = \frac{(\text{Free Phosphate}_{\text{reaction}}, \text{pmol}) - (\text{Background}^*, \text{pmol})}{3000\ \text{pmol}} \times 100\ \%$$

* "Background" is the average free phosphate value of the "PIP_3 Substrate-only" controls. The value from "Enzyme-only" controls is negligible - the absorbance is usually within error range of buffer blank controls.