



Echelon Biosciences Inc.  
675 Arapeen Drive, Suite 302  
Salt Lake City, UT 84108 Telephone  
866-588-0455  
Fax 801-588-0497  
echelon@echelon-inc.com  
www.echelon-inc.com

## Technical Data Sheet

**For research use only**  
Not intended or approved for  
diagnostic or therapeutic use.

**Product Name:** PTEN Phospholipid Phosphatase

**Product Number:** E-3000

**Available Sizes:** 1 Unit  
3 Units  
12 Units

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

**Storage:** Freeze in working aliquots at -70 °C. Repeated freezing and thawing is not recommended.

**Specificity:** PTEN selectively removes phosphate from the 3' position of the inositol ring of PtdIns(3,4,5)P<sub>3</sub> and Ins(1,3,4,5)P<sub>4</sub>.

**Unit Definition:** 1 Unit of PTEN activity is defined as the release of 1 nmol free phosphate per minute using PtdIns(3,4,5)P<sub>3</sub> (PIP<sub>3</sub>, Product # P-3908) as a substrate in a Malachite Green based phosphatase assay (Product # K-1500).

**QA/Product Testing:** Malachite Green Phosphatase Assay

**Related Products:** Malachite Green Assay kit: K-1500, K-1520, K-1530, and K-1540  
PTEN Substrate: P-3908, P-3916, Q-1345, H-39BT, H-39TM, and H-39FL  
Other Lipid Phosphatase: E-1000 (SHIP2)

## References:

- (1) T. Maehama and J.E. Dixon (1998) The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J Biol Chem*, **273**, 13375-8.
- (2) T. Maehama and J.E. Dixon (1999) PTEN: a tumour suppressor that functions as a phospholipid phosphatase. *Trends Cell Biol*, **9**, 125-8.
- (3) M. Tamura, J. Gu, E.H. Danen, T. Takino, S. Miyamoto and K.M. Yamada (1999) PTEN interactions with focal adhesion kinase and suppression of the extracellular matrix-dependent phosphatidylinositol 3-kinase/Akt cell survival pathway. *J Biol Chem*, **274**, 20693-703.
- (4) L.C. Cantley and B.G. Neel (1999) New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. *Proc Natl Acad Sci U S A*, **96**, 4240-5.
- (5) J.M. Penninger and J. Woodgett (2001) Stem cells. PTEN--coupling tumor suppression to stem cells? *Science*, **294**, 2116-8.
- (6) M. Groszer, R. Erickson, D.D. Scripture-Adams, R. Lesche, A. Trumpp, J.A. Zack, H.I. Kornblum, X. Liu and H. Wu (2001) Negative regulation of neural stem/progenitor cell proliferation by the Pten tumor suppressor gene in vivo. *Science*, **294**, 2186-9.
- (7) G. Schwartzbauer and J. Robbins (2001) The tumor suppressor gene PTEN can regulate cardiac hypertrophy and survival. *J Biol Chem*, **276**, 35786-93.
- (8) M.A. Crackower, G.Y. Oudit, I. Kozieradzki, R. Sarao, H. Sun, T. Sasaki, E. Hirsch, A. Suzuki, T. Shioi, J. Irie-Sasaki, R. Sah, H.Y. Cheng, V.O. Rybin, G. Lembo, L. Fratta, A.J. Oliveira-dos-Santos, J.L. Benovic, C.R. Kahn, S. Izumo, S.F. Steinberg, M.P. Wymann, P.H. Backx and J.M. Penninger (2002) Regulation of myocardial contractility and cell size by distinct PI3K-PTEN signaling pathways. *Cell*, **110**, 737-49.
- (9) B. Stiles, M. Groszer, S. Wang, J. Jiao and H. Wu (2004) PTENless means more. *Dev Biol*, **273**, 175-84.
- (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.
- (11) Furnari, F. B., Huang, H. J., and Cavenee, W. K., The phosphoinositol phosphatase activity of PTEN mediates a serum-sensitive G1 growth arrest in glioma cells, *Cancer Res*, **58**, 5002 (1998).
- (12) D. Haas-Kogan, N. Shalev, M. Wong, G. Mills, G. Yount and D. Stokoe (1998) Protein kinase B (PKB/Akt) activity is elevated in glioblastoma cells due to mutation of the tumor suppressor PTEN/MMAC. *Curr Biol*, **8**, 1195-8.
- (13) D.H. Teng, R. Hu, H. Lin, T. Davis, D. Iliev, C. Frye, B. Swedlund, K.L. Hansen, V.L. Vinson, K.L. Gumper, L. Ellis, A. El-Naggar, M. Frazier, S. Jasser, L.A. Langford, J. Lee, G.B. Mills, M.A. Pershouse, R.E. Pollack, C. Tornos, P. Troncoso, W.K. Yung, G. Fujii, A. Berson, P.A. Steck and et al. (1997) MMAC1/PTEN mutations in primary tumor specimens and tumor cell lines. *Cancer Res*, **57**, 5221-5.
- (14) G.B. Mills, Y. Lu and E.C. Kohn (2001) Linking molecular therapeutics to molecular diagnostics: inhibition of the FRAP/RAFT/TOR component of the PI3K pathway preferentially blocks PTEN mutant cells in vitro and in vivo. *Proc Natl Acad Sci U S A*, **98**, 10031-3.
- (15) J. Huang and C.D. Kontos (2002) PTEN modulates vascular endothelial growth factor-mediated signaling and angiogenic effects. *J Biol Chem*, **277**, 10760-6.

# PTEN Malachite Green Assay Protocol

## Buffer and Reagent Preparation:

### 1 x TBS

25 mM Tris-Cl, pH 7.4, 140 mM NaCl, 2.7 mM KCl. Store at room temperature.

### PTEN Malachite Buffer

1 x TBS + 10 mM DTT

Make fresh before use and keep on ice. For 5 mL: Add 50  $\mu$ L 1M DTT to 5 mL 1xTBS

### Phosphate Standards

1mM phosphate standard is included in kit K-1500. Make standard dilutions in PTEN Malachite Buffer following protocol of the kit.

### PIP<sub>3</sub> Substrate

Reconstitute PI(3,4,5)P<sub>3</sub> powder (Product # P-3908) in ddH<sub>2</sub>O to make a 1 mM stock solution. Store at -20 °C between uses. Use 3  $\mu$ L (3,000 pmol) for each assay point.

## Assay:

### PTEN Reaction preparation

Sample	PTEN, ng	PIP <sub>3</sub> , $\mu$ L	PTEN Malachite Buffer
PTEN (Enzyme-only Control)	100	-	to final vol. of 25 $\mu$ L
PIP <sub>3</sub> (Substrate-only Control)	-	3	22 $\mu$ L
PTEN + PIP <sub>3</sub> (Enzyme Reaction)	100	3	to final vol. of 25 $\mu$ L

1. Add phosphate standard solutions 25  $\mu$ L/well in triplicates in a 96-well flat bottom clear plate.
2. Prepare enzyme reactions in triplicate wells according to the table above. Add buffer and enzyme first, then add PIP<sub>3</sub> substrate to start reaction. Seal plate and mix on plate shaker for 30 seconds (if available). Incubate at 37 °C for exactly 15 min.
3. Add 100  $\mu$ L/well Malachite Green solution to each well of controls, phosphate standards, and PTEN reactions. Seal plate and cover with aluminum foil to protect from light. Incubate on a plate shaker (if available) for 15 minutes at room temperature to develop color.
4. Read absorbance at 620 nm.
5. Draw standard curve with Abs. 620 nm as Y axis and phosphate in pmol as X axis.
6. Determine free phosphate in pmol from each reaction or control by interpolation from the standard curve.
7. Calculate PTEN specific activity as follows:

$$\text{Specific activity} = \frac{(\text{Free Phosphate, pmol}) - (\text{Background}^*, \text{ pmol})}{15 \text{ min.} \times 0.1 \mu\text{g PTEN Enzyme}} = \text{___ pmol/min}/\mu\text{g} = \text{___ U/mg}$$

- \* “Background” is the average value of either the “Substrate-only” controls or the “Enzyme-only” controls, whichever is higher.