



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 ELISA  
ELISA Assay for Detection and Quantification  
of PTEN Phosphatase Activity

**Product Number:** K-2300

### Storage and kit contents:

Store kit components at 4°C for up to 6 months.

#### Kit Contents:

- 1-PTEN substrate Plate (pre-coated with PI(3,4,5)P<sub>3</sub> substrate and PI(4,5)P<sub>2</sub> standard curve)
- 3 pellets -PI(4,5)P<sub>2</sub> Detector
- 25 µL HRP conjugate
- 12 mL TMB Solution
- 2 Acetate Plate Sealers

#### Researcher Provides:

- Buffers for reactions and washes
- Source of PTEN Enzyme (cat# E-3000)
- Microplate Reader with capability to read absorbance at 450 nm

### Background and assay overview:

PTEN (Phosphatase and Tensin Homolog deleted on Chromosome 10) is a 3' phosphoinositide phosphatase that converts PI(3,4,5)P<sub>3</sub> 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>,<sup>1</sup> 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 has recently been shown to regulate cancer-associated pathways including VEGF-mediated angiogenesis and others.<sup>15</sup>

Echelon's PTEN ELISA is designed to detect and quantify PTEN phosphatase activity by means of a standard ELISA format, eliminating the need for radioactivity, organic solvents, and thin layer chromatography. The Echelon PTEN activity ELISA directly detects the phosphoinositide product compared to other assays, which detect free phosphate. This eliminates many possible sources of error because inorganic phosphate is the product of many phosphatase enzyme activities, and is found in common buffers and cleaning products. In addition, the PTEN activity ELISA is approximately 100 times more sensitive than free-phosphate assays and can be used with recombinant enzyme, immunoprecipitated enzyme; and potentially, cell lysate or tissue homogenate.

The PTEN Activity ELISA detects PTEN phosphatase activity once the enzyme has converted PI(3,4,5)P<sub>3</sub> (bound to the PTEN substrate plate) to PI(4,5)P<sub>2</sub>. After the PTEN reactions are complete the PI(4,5)P<sub>2</sub> Detector and HRP conjugate are added step-wise. Colorimetric detection is used to quantify the amount of PI(4,5)P<sub>2</sub> produced by PTEN phosphatase activity, compared to a standard curve.

## Preparation Of Solutions:

### PTEN Enzyme Reaction Buffer

10 mM Hepes, 150 mM NaCl, 10 mM DTT, pH 7.2

Note: DTT is not stable in aqueous buffers, add to enzyme reaction buffer immediately before use

### TBS

Tris-buffered Saline, 10 mM Tris, 150 mM NaCl, pH 7.4

### TBS-T

Add 50  $\mu$ L Tween-20 (J.T. baker #X251-07) to 100 mL TBS

### TBS-GS

Add 500  $\mu$ L goat serum (GibcoBRL #0885) to 49.5 mL TBS

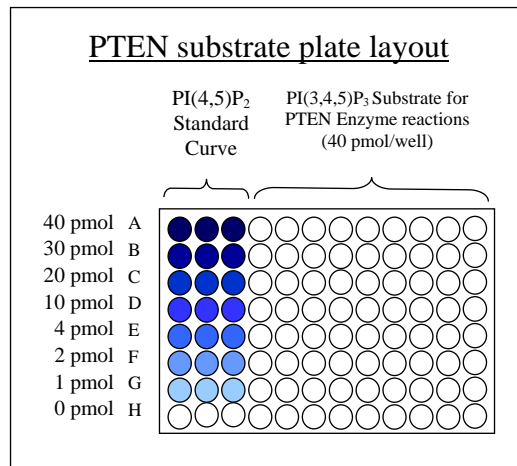
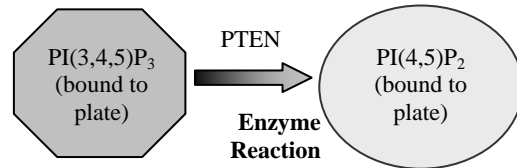
### Peroxidase Stop Solution

0.5 M Sulfuric Acid (dilute concentrated  $H_2SO_4$  1:35 with water)

## Procedure: Enzyme Reactions and Detection

### PTEN Enzyme Reactions

1. **Obtain PTEN enzyme**
2. **Prepare PTEN Enzyme** by diluting in PTEN enzyme reaction buffer. (Suggested concentration for Echelon's PTEN enzyme, 10 –100 nM)
3. **Add enzyme samples** (100  $\mu$ L/well) to PI(3,4,5)P<sub>3</sub> substrate wells (refer to PTEN substrate plate layout). Note: To minimize potential buffer effects add PTEN enzyme reaction buffer (100  $\mu$ L/well) to PI(4,5)P<sub>2</sub> standard curve wells during this step.
4. **Incubate** PTEN substrate plate, covered with an acetate plate sealer, at 37 °C for the appropriate amount of time Note: Incubation times of 15-60 min with recombinant PTEN enzyme from Echelon results in 25%-100% conversion. Time course experiments should be performed to optimize your source of PTEN enzyme.



5. **Stop the enzyme reaction** by discarding enzyme reaction solution then washing each well 3X with 200  $\mu$ L/well TBS-T.

### Detection of Enzyme Reactions

1. **Add PI(4,5)P<sub>2</sub> Detector** to PTEN substrate plate (100  $\mu$ L/well).

Prepare PI(4,5)P<sub>2</sub> Detector: Transfer 1 Detector Pellet to a 15 mL tube and add 10.5 mL TBS-GS. Mix PI(4,5)P<sub>2</sub> Detector then add 100  $\mu$ L to each well of plate. Note: Prepare PI(4,5)P<sub>2</sub> Detector immediately before use; this solution is stable at 4° C for up to 12 hours.

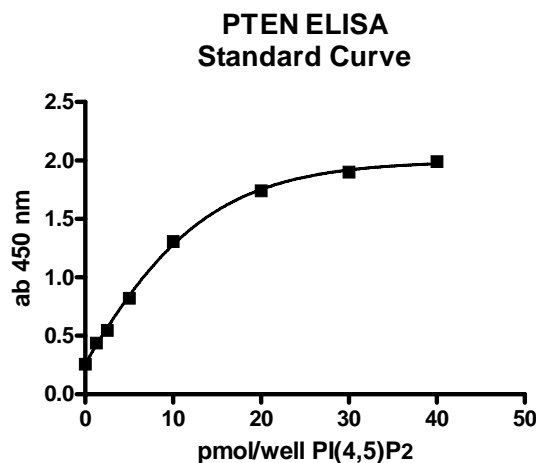
2. **Incubate** PTEN substrate plate, covered with an acetate plate sealer, for 1 hour at room temperature. Note: for optimal results incubate on an orbital plate shaker or with gentle agitation.
3. **Wash** PTEN substrate plate. Remove and discard solution through tapping in a sink or aspiration and wash each well 3X with 200  $\mu\text{L}$ /well TBST. Note: longer wash incubation times and increased number of washes can help reduce background signal.
4. **Add working solution of HRP conjugate** to PTEN substrate plate (100  $\mu\text{L}$ /well).

Prepare working solution of HRP conjugate: Add 21  $\mu\text{L}$  of HRP conjugate to 10.5 mL TBS- GS. Mix then add 100  $\mu\text{L}$  to each well of plate. Note: If using only part of plate, dilute HRP conjugate 1:500 in enough volume of TBS-GS necessary to fill 100  $\mu\text{L}$ /well for the amount of wells used. For example: For 1/3 of the plate add 7 $\mu\text{L}$  HRP conjugate to 3.5 mL TBS-GS.

5. **Incubate:** Repeat step 2.
6. **Wash:** Repeat step 3.
7. **Add TMB** 100  $\mu\text{L}$ /well, incubate plate protected from direct light until the color development is sufficient for photometric analysis (approx. time 15-30 min). Stop reaction with 50  $\mu\text{L}$ /well Peroxidase Stop Solution (0.5M  $\text{H}_2\text{SO}_4$ ).
8. **Read** plate on an absorbance plate reader at 450 nm.

## Data Analysis:

The values obtained for enzyme reactions can be compared to the standard curve to quantify conversion of  $\text{PI}(3,4,5)\text{P}_3$  substrate to  $\text{PI}(4,5)\text{P}_2$ . An example standard curve is shown.



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## Related Products:

	Product	General Description	Detection Mode	Cat. No.
Purified Enzymes	PTEN	Recombinant GST-tagged fusion protein.	See PTEN Activity assays	E-3000
	SHIP2	Recombinant 6X histidine-tagged fusion protein	See SHIP Activity Assays	E-1000
PIP Mass Assays	PI(4,5)P <sub>2</sub> Mass Strip Kit	The PI(4,5)P <sub>2</sub> Mass Strip Kit is designed to quantify PIP <sub>2</sub> , obtained from cell extractions, through a simple lipid-protein overlay experiment.	Chemiluminescent developing solution	K-2900
	PI(3,4,5)P <sub>3</sub> Mass Strip Kit	The PIP <sub>3</sub> Mass Strip Kit is designed to quantify PIP <sub>3</sub> , obtained from cell extractions or PI3K reactions, through a simple lipid-protein overlay experiment.	Chemiluminescent developing solution	K-2400
	PI(3,4,5)P <sub>3</sub> Mass ELISA Assay	Detects extracted PI(3,4,5)P <sub>3</sub> from cells samples. The assay is sensitive to 1 pmol PIP <sub>3</sub> , and requires approximately ~3 x 10 <sup>6</sup> cells per data point depending on the experimental system used.	Absorbance 450 nm	K-2500
PTEN Activity Assays	PTEN FP	Detects PTEN activity in purified and immuno-precipitated samples through detection of PI(4,5)P <sub>2</sub> (Enzyme product of PTEN phosphatase activity)	Flourescence Polarization	K-1600
	Malachite Green	Detects PTEN activity in purified and immuno-precipitated samples through detection of free phosphate (side product of phosphatase activity)	Absorbance 620 nm	K-1500
SHIP /5' Phosphatase Activity Assays	SHIP FP	Detects SHIP activity in purified and immuno-precipitated samples through detection of PI(3,4)P <sub>2</sub> (Enzyme product of SHIP phosphatase activity)	Flourescence Polarization	K-1400
	Malachite Green	Detects SHIP activity in purified and immuno-precipitated samples through detection of free phosphate (side product of phosphatase activity)	Absorbance 620 nm	K-1600
Possible PTEN Substrates	Non-labeled Substrate	Synthesized and provided as a lyophilized powder. Available in long chain (di-C <sub>16</sub> ) or short chain (di-C <sub>8</sub> ) PtdIns(3,4,5)P <sub>3</sub> ; or inositol head group Ins(1,3,4,5)P <sub>4</sub>		P-3916 P-3908 H-3916 Q-1345
	Labeled Substrate	Synthesized and provided as a lyophilized powder. Substrates available with flourescent tag or biotinylated label.		H-39TR H-39BT C-39F6a C-39B6a

Check Echelon's website ([www.echelon-inc.com](http://www.echelon-inc.com)) for other phosphatase/kinase activity assays, purified enzymes, and substrates.