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Technical Data Sheet

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

## PTEN Activity ELISA

### 96-well ELISA Assay for Detection and Quantification of PTEN Phosphatase Activity

**Product Number: K-4700**

#### Materials Provided:

Part #	Description	Quantity
K-4701	PI(4,5)P <sub>2</sub> Coated Strip-well Detection Plate	1 plate
K-4702	PI(3,4,5)P <sub>3</sub> Substrate, diC <sub>16</sub>	30 nmol
K-4703	PI(4,5)P <sub>2</sub> Standard, diC <sub>16</sub>	12 nmol
K-2302	PI(4,5)P <sub>2</sub> Detector	2 x 2.5 µg
K-4704	5x PTEN Reaction Buffer	4 mL
K-GS01	Protein Stabilizer	600 µL
K-SEC2	Secondary Detector	300 µL
K-PTAB	PBS Tablet	1 tablet
K-PBST3	10x PBS-T Buffer	30 mL
K-TMB1	TMB Solution	12 mL
K-STOPt	1 N H <sub>2</sub> SO <sub>4</sub> Stop Solution	10 mL
K-DTT1	DTT	3 x 50 µmol
Incubation Plate	Yellow 96-well polypropylene U-bottom plate	1 plate
Plate Sealers	Clear acetate sheet, 1 side adhesive	3 seals

#### Additional Materials Provided by User

- Source of PTEN Enzyme (cat# E-3000).
- Incubated plate shaker or 37 °C incubator.
- Microplate Reader with capability to read absorbance at 450 nm.

#### Storage

The kit comes in two parts with different storage requirements. Upon receipt store Kit Part 1 at 4 °C and Kit Part 2 at -20 °C. Store prepared reagents as indicated in the protocol.

#### Background and Product Description

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 Activity ELISA is designed to detect and quantify PTEN phosphatase activity by means of a competitive 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 due to the fact that inorganic phosphate is the product of many phosphatase enzyme activities, and is found in common buffers and cleaning products.

The assay is a competitive ELISA in which the signal is inversely proportional to the amount of PI(4,5)P<sub>2</sub> produced. After the PTEN reactions are complete, reaction products are added to the PI(4,5)P<sub>2</sub>-coated microplate and a PI(4,5)P<sub>2</sub> detector protein is then added for competitive binding. A peroxidase-linked secondary detector and colorimetric detection is used to detect PI(4,5)P<sub>2</sub> detector binding to the plate. The colorimetric signal is inversely proportional to the amount of PI(4,5)P<sub>2</sub> produced by PTEN.

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**Assay Protocol** – Please read through entire protocol carefully along with the “Assay Notes” before beginning the assay.

### **Reagent Preparation**

#### **1. PTEN Reaction Buffer:**

Prepare fresh PTEN Reaction Buffer and DTT for use on the day of the assay. Dilute the 5x PTEN Reaction Buffer (K-4704) 5-fold in dH<sub>2</sub>O and supplement with 10 mM DTT (K-DTT1). Each vial of K-DTT1 contains 50 μmol DTT. Add 50 μL dH<sub>2</sub>O for a 1M stock. Once reconstituted, the DTT should be used immediately and any remaining should be discarded.

5 mL of PTEN Reaction Buffer = 1 mL of 5x PTEN Reaction Buffer + 50 μL 1M DTT + 3,950 μL dH<sub>2</sub>O.

#### **2. PBS Buffer:**

Prepare the PBS Buffer by dissolving the provided PBS tablet (K-PTAB) in 200 mL dH<sub>2</sub>O.

#### **3. PBS-T Buffer:**

Prepare the 1x PBS-T Buffer by diluting 30 mL of the 10x PBS-T Buffer (K-PBST3) with 270 mL dH<sub>2</sub>O.

#### **4. PI(3,4,5)P<sub>3</sub> Substrate:**

Equilibrate vial of PI(3,4,5)P<sub>3</sub> Substrate (K-4702) to room temperature. Prepare a 100 μM PI(3,4,5)P<sub>3</sub> Substrate stock solution by adding 300 μL dH<sub>2</sub>O to the vial of PI(3,4,5)P<sub>3</sub> Substrate (K-4702). Vortex for at least 60 seconds to resuspend the lipid. Spin down and place vial at room temperature. Prior to use, dilute the required amount of PI(3,4,5)P<sub>3</sub> Substrate in PTEN Reaction Buffer for a 16 μM (2x conc.) working solution. 1 mL of 16 μM PI(3,4,5)P<sub>3</sub> Substrate can set up 30 PTEN reactions. The unused portion of 100 μM PI(3,4,5)P<sub>3</sub> Substrate stock can be stored at -20 °C for up to 3 months.

1 mL of 16 μM PI(3,4,5)P<sub>3</sub> Substrate = 160 μL of 100 μM PI(3,4,5)P<sub>3</sub> Substrate stock + 840 μL of PTEN Reaction Buffer.

#### **5. PI(4,5)P<sub>2</sub> Standard:**

Equilibrate vial of PI(4,5)P<sub>2</sub> Standard (K-4703) to room temperature. Prepare a 40 μM PI(4,5)P<sub>2</sub> Standard stock solution by adding 300 μL dH<sub>2</sub>O to the vial of PI(4,5)P<sub>2</sub> Standard (K-4703). Vortex for at least 60 seconds to resuspend the lipid. Spin down and place vial at room temperature. The unused portion of 40 μM PI(4,5)P<sub>2</sub> Standard stock can be stored at -20 °C for up to 3 months.

### **PTEN Reaction and Incubation**

1. Isolate or prepare PTEN according to usual protocols. See attached support protocol for immunoprecipitation of PTEN from cells. Prior to use, dilute the required amount of PTEN to a 2x concentration in the PTEN Reaction Buffer.
2. PTEN reactions can be set up in micro centrifuge tubes or in the yellow Incubation Plate (using recombinant PTEN enzyme only). Please read assay notes at the end of the protocol first.
  - a. For each 60 μL PTEN reaction (for duplicate assay points): combine 30 μL of the 16 μM PI(3,4,5)P<sub>3</sub> Substrate (480 pmol) and 30 μL of the PTEN (2x conc.). You may also include an Enzyme Only control by replacing 30 μL of Substrate with 30 μL of PTEN Reaction Buffer.
  - b. Seal the PTEN reactions and let them proceed **without vigorous shaking** for a certain time, usually 1-4 hours at **37 °C or RT**  
**Note:** If using PTEN bound to beads, light agitation or slow rotation will be necessary to keep beads in suspension.
3. Stop each 60 μL reaction.
  - a. If PTEN enzyme is bound to beads, centrifuge to separate the beads and transfer reaction supernatant to clean tubes.
  - b. For recombinant PTEN, heat the reaction for 3 minutes at 95 °C to stop the reaction.
4. Add an additional 62 μL of PTEN Reaction Buffer to each stopped reaction for a total of 122 μL.  
**Note:** Reaction products can be stored at -20 °C for up to a week. ELISA Detection can be run on another day.

We suggest that standards and controls be run in duplicate or triplicate. The Incubation Plate layout shown on the next page gives an example plate layout with triplicate standards and controls.

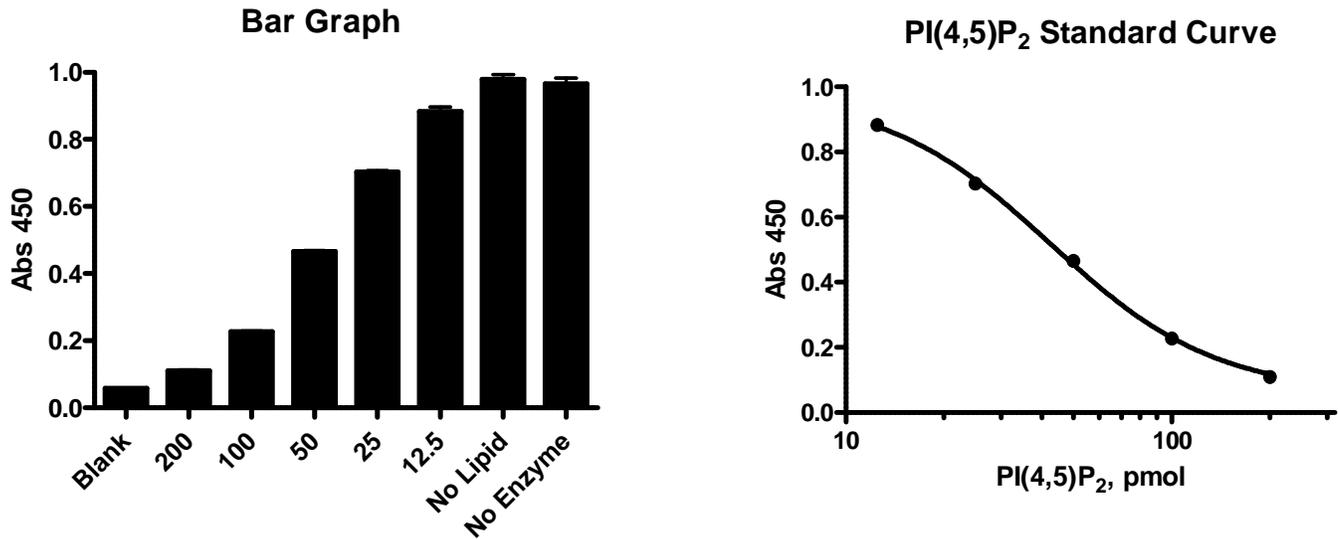
#### **5. Prepare PI(4,5)P<sub>2</sub> Standards and Controls:**

- a. From the 40 μM PI(4,5)P<sub>2</sub> Standard stock prepared earlier, prepare a 4 μM working solution by adding 40 μL of the 40 μM stock solution to 360 μL of the PTEN Reaction Buffer.
- b. Make four, 2-fold serial dilutions from the 4 μM PI(4,5)P<sub>2</sub> stock with PTEN Reaction Buffer.  
e.g. Each 400 μL dilution = 200 μL previous dilution + 200 μL PTEN Reaction Buffer.
- c. Prepare a 4 μM working solution of PI(3,4,5)P<sub>3</sub> Substrate for the “No Enzyme” control by diluting 8 μL of the 100 μM PI(3,4,5)P<sub>3</sub> Substrate stock solution with 192 μL PTEN Reaction Buffer.
- d. Pipet 60 μL/well of each PI(4,5)P<sub>2</sub> Standard solution to rows A through E of the Incubation Plate.
- e. Pipet 60 μL/well of the 4 μM PI(3,4,5)P<sub>3</sub> Substrate solution to the No Enzyme control wells in row F of the Incubation Plate.
- f. Pipet 60 μL/well of PTEN Reaction Buffer to the No Lipid control wells in row G of the Incubation Plate.

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## Results



PTEN activity can be estimated by comparing the absorbance values from the wells containing enzyme reaction products to the values in the standard curve. Plot the absorbance values obtained vs. log of PI(4,5)P<sub>2</sub> in pmol per standard to generate a standard curve using sigmoidal dose-response (variable slope) correlation. Determine the PI(4,5)P<sub>2</sub> level in pmol by interpolation from absorbance values obtained from the enzyme reactions. PTEN activity in your samples can be estimated by the percentage conversion from initial 200 pmol of PI(3,4,5)P<sub>3</sub> per assay point.

### Assay Notes:

1. Optimization of the PTEN reactions by enzyme amount, reaction time and temperature may be required.
2. The provided 5x PTEN Reaction Buffer (K-4704) must be used for the PTEN reactions.
3. Vigorous shaking will kill PTEN enzyme activity. When using PTEN bound to beads, some agitation will be necessary to keep beads in suspension. When using recombinant PTEN there is no need to shake the reaction.
4. PTEN reactions can be carried out at either 37 °C or at room temperature. When performing reactions at room temperature, the incubation time should be increased.
5. The amount of enzyme to use per PTEN reaction will vary according to your individual experiment. Whether you are using purified PTEN or enzyme immunoprecipitated from cell lysate, you will need to try reactions using different amounts of enzyme to determine the optimum condition. When using purified recombinant PTEN from Echelon (Cat# E-3000), enzyme concentration of 0.5-2 ng/μL is suggested as a starting point. In testing, we found that enzyme immunoprecipitated from cell lysate containing 1-5 mg cellular protein is usually sufficient for each reaction.
6. Beads need to be removed from enzyme reactions before reactions are stored or detected.
7. Step 1 of the “Detection” protocol requires an incubation at 37 °C with shaking. If an incubated plate shaker is not available, then the incubation can be done at 37 °C without shaking for a period of 2 hours.
8. The detection plate is composed of 12 of 8-well strips. Unused strip wells should be removed from the plate frame and stored in a clean sealable plastic bag at 4 °C. Save the plate frame after assay for future use of the remaining strip wells.
9. Never let the detection plate dry out after the ELISA assay has started. Always prepare the next solution needed before discarding the current one from wells in use.

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## Support Protocol

### Immunoprecipitation of PTEN From Cells

The following support protocol for immunoprecipitation of PTEN from cells has been validated for use with the PTEN Activity ELISA. Further optimization of this protocol and/or different IP protocols can be used to IP PTEN from cells depending on the needs and experience of the user.

<b>Materials Needed:</b>	<b>Company</b>	<b>Catalog Number</b>
1.5 mL Centifuge Tubes	N/A	N/A
Lysis Buffer (25 mM Tris pH 8.0, 150 mM NaCl, 1% NP-40, 1mM EDTA, 5% Glycerol)	N/A	N/A
Protease Inhibitor Cocktail	Sigma	P8340
Protein A Agarose Beads	Pierce	20333
PTEN (D4.3) XP™ Rabbit mAb	Cell Signaling	9188
PTEN Reaction Buffer	Echelon	K-4704
PBS	N/A	N/A

This protocol is written for a 100 mm dish of NIH 3T3 cells (90% confluent) for about 15-20 mg/mL of cellular protein. To maximize PTEN activity; keep all solutions ice cold, carry out all reactions on ice or at 4°C, and use a centrifuge that is equilibrated at 4°C.

### Reagent Preparation:

#### 1. Lysis Buffer:

Prepare the Lysis Buffer according to the recipe listed on the materials. Add the protease inhibitor cocktail fresh at a 1:100 dilution. Place buffer on ice and chill until ice cold.

#### 2. PTEN Reaction Buffer:

See preparation instructions in the “Reagent Preparation” section in the main protocol. PTEN Reaction Buffer must be made fresh just prior to reaching step 11 in this protocol. Keep buffer at room temperature once prepared.

### Cell Lysis:

1. Place cell culture dish on ice and wash cells twice with ice cold PBS.
2. Add 0.5 mL of ice cold Lysis Buffer to cells. Scrape cells and transfer mixture into a cooled 1.5 mL centrifuge tube.
3. Incubate cells for 15 minutes with constant agitation at 4°C.
4. Centrifuge cells for 10 minutes at 14,000 x g to pellet cells.
5. Transfer supernatant to a fresh, cooled 1.5 mL centrifuge tube and place on ice. Discard pellet.

### Immunoprecipitation:

6. Transfer 400 µL of the cell lysate to a fresh, cold, 1.5 mL centrifuge tube.
7. Add 8 µL of the anti-PTEN antibody (Cell Signaling) to the lysate. Incubate 3 hours to overnight at 4°C with agitation.
8. Add 60 µL of the 50% Protein A agarose beads to the mixture and incubate 2-3 hours at 4°C.
9. Briefly centrifuge to pellet beads. Discard the supernatant.
10. Wash the bead complex three times with ice cold PBS. Centrifuge and discard solution after each wash.
11. Wash once with PTEN Reaction Buffer.
12. Resuspend bead complex in 30 µL of PTEN Reaction buffer.
13. Proceed immediately with the PTEN reactions by adding 30 µL of the 16 µM PI(3,4,5)P<sub>3</sub> Substrate to the bead complex. (See the “PTEN Reaction and Incubation” section in the main protocol).