



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 Fluorescence Polarization Activity Assay
Product Number: K-1600

Storage: Some components are temperature and light sensitive. Store unopened kit at -20°C until use.

I. Components (384 assay points per kit)

- Kit includes:**
- 2 blue capped tubes of PIP₃ Substrate, 1100 μL /tube of 10 μM stock
 - 1 green capped tube of PI(4,5)P₂ Standard, 330 μL of 10 μM stock
 - 16 vials PIP₂ Grip (PI(4,5)P₂ detector), 400 reactions
 - 1 red capped tube of PI(4,5)P₂ Probe, 55 μL of 2 μM stock, 40X
 - Phosphate buffered saline (PBS) tablet
 - 1 yellow capped tube of Enzyme Reaction Buffer 10X, 300 μL of 3M Tris-HCl, pH 8.0, and 300mM dithiothreitol.
 - 1 black 384-well plate, nonreactive and nonbinding surface

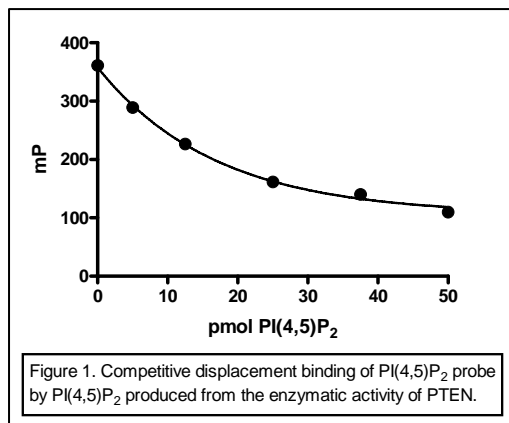
Researcher must provide: Source of phosphatase Enzyme

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TDS K-1600 Rev: 2 (3/11/04)

- Acetate plate sealers or equivalent
- Fluorescence plate reader capable of Fluorescence Polarization detection with appropriate filters for red fluorophores (for example, 550 nm excitation/580 nm polarizing emission filters)

II. Background



The Echelon PTEN assay is a competitive assay. After the Phosphatase reactions are complete, reaction products are mixed with a PI(4,5)P₂ detector protein (PIP2 Grip) and the fluorescent PI(4,5)P₂ probe. Polarization (mP) values decrease as PI(4,5)P₂ detector binding to the Probe is displaced by PI(4,5)P₂ produced by dephosphorylation of PI(3,4,5)P₃. Figure 1 shows typical results produced by increasing PI(4,5)P₂ on polarization values.

III. Reagent Preparation and Storage:

PIP₃ Substrate: Two blue capped tubes containing 1,100 μL/tube of a 10 μM solution of diC₈ PI(3,4,5)P₃, enough substrate for 400 assay points and 4 standard curves, using 50 pmols per assay point. Store frozen at -20 °C. Multiple freeze-thaw cycles do not affect stability.

PI(4,5)P₂ Standard: One green capped tube containing 330 μL of 10 μM solution of diC₈ PI(4,5)P₂, enough for 4 standard curves. Store frozen at -20 °C. Multiple freeze-thaw cycles do not affect stability.

4,5)P₂ detector): Sixteen vials containing enough pelleted PI(4,5)P₂ detector for a total of 400 assay points. Store at -20 °C. **This reagent is not stable at 4 °C.** Add 125 μL PBS per vial for a 2 μM working solution.

Mix only enough reagent to be used in one day. (For an entire plate you can add all sixteen pellets to one tube before adding 2,000 μl volume) **Keep this reagent on ice at all times.**

PI(4,5)P₂ Probe: One red capped tube containing 55 μ L of 2 μ M solution of BODIPY[®] TMR-labeled probe. Store frozen at -20 °C. **IMPORTANT: Minimize exposure of this reagent to light.** Dilute stock solution of probe 1:40 in PBS for a 50 nM working solution immediately prior to assay.

PBS buffer: Dissolve PBS tablet in 200 mL of purified water. The final composition and concentrations are: 10 mM phosphate, 2.7 mM KCl, 137 mM NaCl. This PBS buffer may be stored at room temperature for 1 month.

Enzyme Reaction Buffer 10X: One yellow capped tube containing 300 μ L of 10X enzyme reaction buffer. Store frozen at -20 °C. **This reagent is not stable at 4 °C.** Dilute 10X stock buffer to 1X working buffer by adding 250 μ L to 2250 μ L distilled water. The working concentration is 300 mM tris-HCl, pH 8.0, 30 mM dithiothreitol (DTT). The final concentration in Enzyme reaction is 100 mM Tris-HCl, pH 8.0, 10 mM DTT. **Keep this reagent on ice at all times.**

NOTE: Bring plate to room temperature prior to assay.

IV. Assay Setup-Standards and Controls:

Set up a standard conversion curve of PI(4,5)P₂ and PI(3,4,5)P₃ to allow determination of the amount of PI(4,5)P₂ produced. A suggested curve is outlined in the table below. We suggest setting up a probe alone control standards be run in duplicate or triplicate.

Percent Conversion PI(3,4,5)P ₃ to PI(4,5)P ₂	100%	75%	50%	25%	10%	0%
Amount of 10 μ M PI(3,4,5)P ₃ to add	0 μ l	25 μ l	50 μ l	75 μ l	90 μ l	100 μ l
Amount of 10 μ M PI(4,5)P ₂ to add	100 μ l	75 μ l	50 μ l	25 μ l	10 μ l	0 μ l

Enough PI(4,5)P₂ and PI(3,4,5)P₃ are included to run six standard conversion curves in triplicates.

V. PTEN reaction and detection of PTEN activity

The amounts and exact conditions for enzyme activity will depend on the characteristics and source of the enzyme used in each specific application. The following protocol has been used at Echelon to detect the activity of a recombinant GST-tagged PTEN and is given as a guideline only. If you have an established protocol for detecting phosphatase activity, your protocol may be used as a substitute. Note: the assay will not tolerate the presence of BSA in buffers. We suggest running several assay points for each enzyme reaction and control.

Add to each well of the black 384-well plate in the following order:

1. 5 μ L/well working stock (1X) Enzyme Reaction Buffer.
2. 5 μ L /well of Enzyme (diluted in H₂O)
or distilled H₂O (Probe Alone and Standard Conversion Curve controls)
3. 5 μ L/well of PI(3,4,5)P₃ Substrate (10 μ M = 50 pmol/well)
or Standard Conversion Curve
or distilled H₂O (Probe Alone control)

Incubate at room temperature (or 37 °C) for an appropriate period of time, depending on the activity of your enzyme. The exact amount of enzyme and conditions of incubation will vary with different enzyme preparations and will need to be optimized, by the user, for each specific application.

Then add to all wells used:

1. 5 μ L/well PIP₂ Grip (PI(4,5)P₂ detector), except for probe alone control, use PBS
2. 5 μ L/well PI(4,5)P₂ Probe (50 nM).

Each well should have a final volume of 25 μ L. Seal the plate, protect from light, and incubate in a dark location at room temperature for 15 minutes to one hour.

Measure the fluorescence polarization using an appropriate microplate reader with a filter set compatible with BODIPY[®] TMR dye. (550 nm excitation/580 nm polarizing emission filters give satisfactory results.).

The values obtained for enzyme reactions can be compared to the standard curve to quantify conversion of PI(3,4,5)P₃ substrate to PI(4,5)P₂.

NOTE: The sensitivity of the assay and the amount of substrate, detector, and fluorescent probe required for each assay point may vary depending on the specific fluorescence polarization plate reader you are using. Please contact your plate-reader manufacturer or Echelon for assistance in modifying the protocol for your use.

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