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Technical Data Sheet

For research use only

Not intended or approved for
diagnostic or therapeutic use.

Product Name: PIP₃ Mass Strip Kit™

Product Number: K-2400

General Description: Phosphoinositide 3-kinase (PI3-K), a ubiquitously expressed lipid kinase, phosphorylates PI(4,5)P₂ at the 3-hydroxyl of the inositol ring producing PtdIns(3,4,5)P₃ (PIP₃). PIP₃ has been shown to be an important lipid second messenger with key roles in fundamental cellular responses such as proliferation and survival and are increasingly attractive targets for drug development, especially in the disease areas of inflammation and cancer.

The PIP₃ Mass Strip Kit is designed to quantify PIP₃, obtained from cell extractions or PI3K reactions, through a simple lipid-protein overlay experiment.

Kit Contents: PIP₃ Strip: 3 nitrocellulose membrane strips pre-spotted with PIP₃ standards and space for spotting 6 unknown samples.

PIP₃ Dectector: 20 µL PIP₃ specific binding protein

Secondary Detector: 450 µL of peroxidase-conjugated Secondary Detector.

Researcher Provides: Lipid sample, buffers, chemiluminescent developing solutions, imaging station, and/or film.

Storage: Store at 2-8°C. Product is moisture and light sensitive.

Format: PIP₃ Strips have a diagonal cut on the top left corner and Ponceau S* staining to assist in orientation. See template below for location of prespotted PIP₃ standard curve and controls, as well as the locations for spotting your samples.

PIP₃ Strip Template:

Sample # 1	○ ○	20 pmol PtdIns(3,4,5)P ₃
Sample # 2	○ ○	10 pmol PtdIns(3,4,5)P ₃
Sample # 3	○ ○	5 pmol PtdIns(3,4,5)P ₃
Sample # 4	○ ○	2.5 pmol PtdIns(3,4,5)P ₃
Sample # 5	○ ○	1.25 pmol PtdIns(3,4,5)P ₃
Sample # 6	○ ○	Solvent Blank

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Lipid Extraction Protocol: Example Protocol for the Extraction of PI(3,4,5)P₃ from cells
Procedure works well with 5 x 10⁶ cells (75 cm² flask at 60% confluency).
Larger or smaller amounts of cells require proportional adjustments of volumes. The amount of cells necessary, for PIP₃ quantification, needs to be determined for each cell type.

1. Collect cells: Aspirate media from cells. Add 4 mL cold 0.5 M TCA. Incubate on ice for 5 min. Scrape cells and transfer into 15 mL tube. Centrifuge at 1500 RPM for 5 min at 4°C. Discard supernatant.
2. Wash pellet: Add 3 mL 5% TCA/1 mM EDTA to cell pellet. Vortex. Centrifuge at 1500 RPM for 5 min at 4°C. Discard supernatant. Repeat wash.
3. Extract neutral lipids: Add 3 mL MeOH: CHCl₃ (2:1) to pellet. Incubate 10 min at room temperature. Vortex 3 times during incubation period. Centrifuge at 1500 RPM for 5 min at 4°C. Discard supernatant. Repeat extraction.
4. Extract Acidic Lipids: Add 2.25 mL CHCl₃: MeOH: 12 M HCl (40:80:1) to pellet. Incubate 15 min at room temperature. Vortex 4 times during incubation period. Centrifuge at 1500 RPM for 5 min at 4°C. Transfer supernatant to a new 15 mL tube.
5. Phase Split: To supernatant, add 0.75 mL CHCl₃ before adding 1.35 mL 0.1 M HCl. Vortex. Centrifuge at 1500 RPM for 5 min at 4°C to separate organic and aqueous phases. Transfer organic (lower) phase to 1.5 mL tube, and dry in a vacuum dryer.

Solutions for Extraction: 0.5 M TCA: For 50 mL add 4.08g TCA and bring to volume with H₂O.
5% TCA with 1 mM EDTA: For 50 mL add 18.61 mg EDTA, 2.5 g TCA to bring to volume with H₂O.
MeOH: CHCl₃ (2:1): For 50 mL add 33.3 mL MeOH, and 16.7 mL CHCl₃
MeOH: CHCl₃: 12 M HCl (80:40:1): For 50 mL add 33 mL MeOH, 16.5 mL CHCl₃, and 0.41 mL 12 M HCl
0.1 M HCl: For 50 mL add 0.41 mL 12 M HCl to 49.39 mL H₂O.

References: Grey, H. Olsson, I.H. Batty, L. Priganica, C.P. Downes: Nonradioactive methods for the assay of phosphoinositide 3-kinases and phosphoinositide phosphates and selective detection of signaling lipids in cell and tissue extracts. *Analytical Biochemistry* 313 234-245 (2003).

Detection Protocol:

For Detection and Quantification of PIP₃

1. To dried down lipid samples add 10 μ L in CHCl₃: MeOH: H₂O (1: 2: 0.8). Vortex samples for 30 seconds before sonicating in water bath sonicator 5 min with ice. Incubate samples at room temperatures for 30-60 min. Vortex samples again for 30 seconds before spotting onto a PIP₃ Strip. Allow to dry 5 min at room temperature before proceeding to the next step.

Note: Spot only 1 μ L volume at a time per spot. If larger volumes are necessary spot 1 μ L and then allow to dry before spotting again in the same area.

Samples during sonication steps must be kept cool.

2. Block PIP₃ Strip with 5-10 mL/strip PBS-3% BSA. Gently agitate for 1 hour at room temperature.

Note: This step can be incubated overnight at 4° C.

3. Prepare PIP₃ Detector solution by adding 5 μ L PIP₃ Detector to 5 mL PBS-3% BSA. Discard blocking solution from PIP₃ Strips and add PIP₃ Solution. Gently agitate for 45 min at room temperature.

Note: This step can be incubated overnight at 4° C.

4. Discard PIP₃ Detector solution from PIP₃ strip and wash strip three times with gentle agitation in 15 mL PBS-T for 5 min/wash.

5. Prepare Secondary Detector solution by adding 125 μ L Secondary Detector to 5 mL PBS-3% BSA. Discard PIP₃ Detector solution from PIP₃ Strips and add Secondary Detector solution. Gently agitate for 45 min at room temperature.

6. Discard Secondary Detector solution from strips and wash strip three times with gentle agitation in 15 mL PBS-T for 5 min/wash.

7. Detect with chemiluminescent developing solution and visualize with imaging station.

Buffers:

PBS: 10 mM phosphate, 2.7 mM Potassium Chloride, 137 mM Sodium Chloride pH 7.4 at 25°C.

PBS-T: PBS with 0.1% Tween-20.

PBS-3% BSA: 100 mL PBS with 3 g fatty acid free BSA.

References:

Xiaoqing Tang, Aimee M. Powelka, Neil A. Soriano, Michael P. Czech, and Adilson Guilherme: PTEN, but Not SHIP2, Suppresses Insulin Signaling through the Phosphatidylinositol 3-Kinase/AKT Pathway in 3T3-L1 Adipocytes. *Journal of Biological Chemistry* 280 22523-22529 (2005).

*Final concentration of 0.1% (v/v) Ponceau S was added for accuracy during spotting.

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