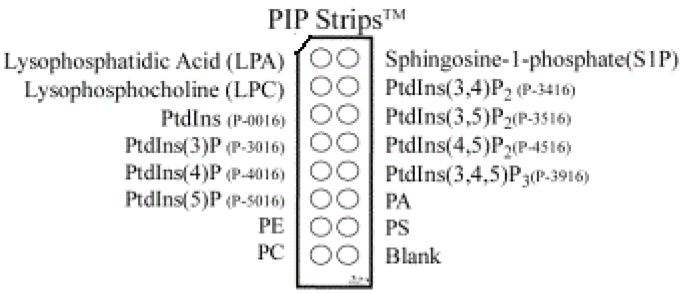
FT-P5802A

PIP Strips™

Product Description

PIP Strips allow to determine if proteins of interest interact specifically with one or more of the phosphoinositides by performing a simple protein.lipid overlay experiment. Lipids are immobilized on a 2 x 6 cm nitrocellulose membrane. Lipid-binding protein are captured from samples and then detected by an antibody against the protein of interest, or the protein may be labelled (biotinylation, enzyme, fluorescence, radioactivity).



Product : Nitrocellulose-immobilized phospholipids at 100 pmol per spot
Each strip has 100 pmol of PtdIns, PtdIns(3)P, PtdIns(4)P, PtdIns(5)P, PtdIns(3,4)P_2, PtdIns(3,5)P_2, PtdIns(4,5)P_2, PtdIns(3,4,5)P_3, phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatic acid (PA) spotted onto a nitrocellulose membrane.
PIP Strips™ also include 100 pmol of the following: lysophosphatidic acid (LPA), lysophosphocholine (LPC), and sphingosine-1-phosphate (S1P).

The top left corner has a diagonal cut. The first lipid is spotted near the diagonal.
Final concentration of 0,1% (v/v) Ponceau S was added for accuracy during spotting.

Cat number : FP-P5802A-2 units
FP-P5802B-10 units

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

Suggested Usage : Lipid-protein overlay and antibody dot blot.

Directions for use

Guidelines for use

1. Block the membrane in TBS-T+3% fatty acid free BSA (InterBiotech # AH642) and gently agitate for one hour at room temperature. See information about alternate blocking/protein dilution solutions in the notes below.
2. Incubate the membrane with 0.5 µg/ml protein of interest in TBS-T+3% fatty acid free BSA for 1-4 hours at room temperature or at 4°C overnight. See comments on protein concentration in the notes below.
3. Wash the membrane with TBS-T+3% fatty acid free BSA three times with gentle agitation for ten minutes each. This wash step should be repeated between all incubation steps and before detection.
4. Additional incubation and wash steps as needed. For detection of GST-fusion protein binding, we recommend incubation with anti-GST monoclonal antibody (#GK2300) at 1:15000 in TBS-T+3% BSA.
5. Detect the bound protein using your method of choice e.g., UptiLight™ detection from Uptima (# UP99619A).

Solutions

TBS-T:	**TBS-T + 3% BSA:**	**TBS-T + 0.1% ovalbumin:**
10 mM Tris 150 mM NaCl pH to 8.0 with HCL 0.1% (v/v) Tween-20	100 ml TBS-T 3 g fatty acid free BSA	100 ml TBS-T 0.1 g ovalbumin

Notes:

For each incubation step, if you have sufficient solution volume, dunk the strip in each liquid in one motion. This method of wetting the strips is better than allowing the liquids to wick through the strip. Make sure that the membrane is completely wetted during the blocking step, and do not allow it to dry between steps.

0,5 µg/ml is given as a starting concentration for detection of interaction of proteins and PIP_ns. The optimum amount of protein that will give a specific signal can vary considerably depending on the interaction being studied. If you find that your protein gives a high background, or interacts with multiple PIP_ns instead of showing the specificity you expect, we recommend that you decrease the amount of protein used in the initial incubation step to 0,05 or 0,005 µg/ml.

Troubleshoot high background the same as you would for Western blots. Changing the composition of the blocking solution can sometimes improve problems with high background. For some proteins, we have observed that using 0,1% ovalbumin (#UPR5851A) in TBS-T as a replacement or 4% non-fat dry milk in TBS-T for TBS-T +3% BSA can result in lowered background and increased specificity for PIP binding. If you use BSA, use a fatty acid free variety.

*We **do not** recommend stripping and reprobing the strips or arrays in the same manner in which a Western/protein blot can be stripped and reprobed. The limited stability of the individual lipid spots following such treatment has been adressed.*

Control (P5802b)
PIP Grip™ pellets contain phosphoinositide-specific proteins used as control reagents for use with our PIP Strips™, PIP Plate™ and PIP Array™, and other bioassays. They are easily reconstituted in water or buffered solutions, and each pellet contains enough protein for use on one PIP Strip or PIP Array™. PIP Grip™ pellets are GST tagged and can be detected with commercial anti-GST antibodies.

—Biological Activity: PLC(delta) - Specificity for PI(4,5)P2

—Use: Store at -20 °C; Easily reconstituted in water or buffered solutions.
Pellets contains enough protein for one PIP Strip™ or PIP Array™.

Ordering information

Catalog size quantities and prices may be found at http://www.fluoprobes.com

For any information, please ask Fluoprobes / Interchim; Hotline : +33(0)4 70 03 73 06

Phospholipid source: Synthetic diC-16 phosphoinositides are produced at Echelon Biosciences Inc.

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