Revised: 19–February–2004

PIP Strips™, PIP MicroStrips™, SphingoStrips™ and PIP Array™ Membranes

Quick Facts

Storage upon receipt:

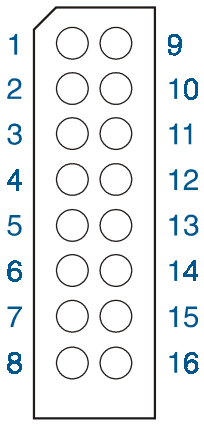
- 2–6°C
- Do not freeze
- Desiccate
- Protect from light

Introduction

Protein domains that specifically bind phosphoinositides have emerged as major determinants in localizing proteins to their site of function.[1–4] These phosphoinositide-binding motifs, which include the C2 (PKC conserved region 2), PH (Pleckstrin homology), FYVE (Fab1p/YOTP/Vac1p/EEA1), ENTH (Epsin NH_2-terminal homology) and PX (Phox homology) domains, are found in proteins implicated in a diverse array of cellular processes, such as actin cytoskeletal organization, cell growth regulation, control of gene expression, protein transport, exocytosis and endocytosis. Through localized phosphoinositide biosynthesis within the cell, proteins containing these lipid-recognition domains can be directed to functionally appropriate sites. Sphingolipid–protein interactions are involved in physiological and pathological processes, including signal transduction mediated by G-protein–coupled receptors (GPCRs),[5,6] induction of apoptosis [7] and amyloid fibril formation.[8] In collaboration with Echelon Biosciences, Molecular Probes offers PIP Strips™, PIP MicroStrips™, PIP Array™ and SphingoStrips™ membranes, which are designed for the identification of proteins possessing phosphoinositide or sphingolipid recognition domains and for analysis of their lipid-binding specificities (Table 1).

Table 1. *Molecular Probes' PIP Strips, PIP MicroStrips, SphingoStrips and PIP Array membranes.*

Product	Unit Size	Catalog Number
PIP Strips™ membranes	2 strips	P23750
	10 strips	P23751
PIP MicroStrips™ membranes	10 strips	P23752
SphingoStrips™ membranes	10 strips	S23753
PIP Array™ membranes	2 strips	P23748
	5 strips	P23749



Spot #	PIP Strips * and PIP MicroStrips †	SphingoStrips *
1	Lysophosphatidic acid	Sphingosine
2	Lysophosphatidylcholine	Sphingosine 1-phosphate
3	Phosphatidylinositol (PtdIns)	Phytosphingosine
4	PtdIns(3)P	Ceramide
5	PtdIns(4)P	Sphingomyelin
6	PtdIns(5)P	Sphingosylphosphocholine
7	Phosphatidylethanolamine	Lysophosphatidic acid
8	Phosphatidylcholine	Myriocin
9	Sphingosine 1-phosphate	Monosialoganglioside G_{M1}
10	PtdIns(3,4)P_2	Disialoganglioside G_{D3}
11	PtdIns(3,5)P_2	Sulfatide
12	PtdIns(4,5)P_2	Sphingosylgalactoside (psychosine)
13	PtdIns(3,4,5)P_3	Cholesterol
14	Phosphatidic acid	Lysophosphatidylcholine
15	Phosphatidylserine	Phosphatidylcholine
16	Blank	Blank

* 100 picomoles of lipid per spot. † 20 picomoles of lipid per spot.

Figure 1. *Layout of PIP Strips, PIP MicroStrips and SphingoStrips membranes. The dimensions of PIP Strips and SphingoStrips membranes are 2 cm × 6 cm. The dimensions of PIP MicroStrips membranes are 1 cm × 2 cm.*

PIP Strips and PIP MicroStrips membranes facilitate the analysis of phosphoinositide protein interactions by protein–lipid overlay assays.[9,10] Proteins may be detected using standard Western blot procedures in conjunction with our high-performance alkaline phosphatase– and horseradish peroxidase–mediated signal generation systems. PIP Array membranes provide eight different phospholipids arrayed in amounts from 100 to 1.6 picomoles, allowing assessment of the strength of protein binding, in addition to lipid specificity.

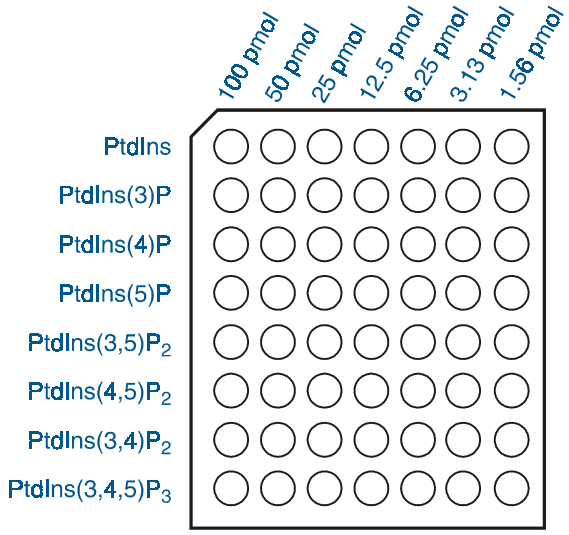


Figure 2. *Layout of immobilized phosphoinositides on PIP Array membranes. The dimensions of PIP Array membranes are 4 cm × 4 cm.*

Materials

Contents

- PIP Strips membranes are provided in sets of two (P23750) or ten (P23751) strips. Each 2 cm × 6 cm nitrocellulose membrane contains 100 pmol samples of 15 different phospholipids and a blank sample (Figure 1).
- PIP MicroStrips membranes are provided in a set of ten 1 cm × 2 cm nitrocellulose strips (P23752). Each strip contains 20 pmol samples of 15 different phospholipids and a blank sample (Figure 1).
- PIP Array membranes are provided in sets of either two (P23748) or five (P23749) arrays. Each 4 cm × 4 cm array contains eight different phospholipids arrayed in amounts from 100 to 1.6 pmol (Figure 2).
- SphingoStrips membranes are provided in packages of 10 strips (S23753). Each 2 cm × 6 cm nitrocellulose membrane contains 100 pmol samples of 15 different lipids and a blank sample (Figure 1).

Storage

Store PIP Strips, PIP MicroStrips, PIP Array and SphingoStrips membranes at 2–6°C, protected from light and moisture. DO NOT FREEZE.

Materials Needed but Not Provided

The following solutions will be needed to perform the assay protocol:

- **TBS-T:** 10 mM Tris–HCl, pH 8.0, 150 mM NaCl, containing 0.1% (v/v) Tween® 20 detergent
- **TBS-T + 3% BSA:** 100 mL of TBS-T plus 3 g of fatty acid–free bovine serum albumin (BSA)

Experimental Protocol

Immerse the membrane in each solution, using one motion, for each incubation step. This method is preferred over allowing the liquids to wick through the membrane. Make sure that the membrane is completely wetted during the blocking step, and do not allow it to dry between steps.

1. Block the membrane. Use TBS-T + 3% fatty acid–free BSA, and gently agitate for one hour at room temperature (note **A**).

2. Incubate the membrane. Incubate using 0.5 µg/mL of the desired protein (note **B**) in TBS-T + 3% fatty acid–free BSA for 1–4 hours at room temperature, or at 2–6ºC overnight.

3. Wash the membrane. Wash the membrane with TBS-T + 3% fatty acid–free BSA three times using gentle agitation for ten minutes each time. This wash step should be repeated between all incubation steps and before detection.

4. Repeat incubation and wash steps. Perform additional incubation and wash steps as needed.

5. Detect the protein. Detect the bound protein using your method of choice (note **C**). For detection of GST fusion proteins, our highly purified rabbit polyclonal anti–GST antibody (A5800) can be used in conjunction with horseradish peroxidase– or alkaline phosphatase–labeled secondary antibodies (G21234, G21079).

Notes

[A] Troubleshoot high background the same as you would for Western blots. To potentially improve problems with high background, change the composition of the blocking solution. For some proteins, using 0.1% ovalbumin in TBS-T instead of TBS-T + 3% fatty acid–free BSA can result in lowered background and increased specificity for phosphoinositide or sphingolipid binding.

[B] A concentration of 0.5 µg/mL is given as a starting point for detecting the interaction of proteins and phosphoinositides. The optimum amount of protein that will give a specific signal can vary considerably depending on the interaction being studied. If a particular protein gives a high background, or interacts with multiple phosphoinositides or sphingolipids instead of showing the expected specificity, it may help to reduce the protein concentration used in the incubation step to 50 ng/mL or 5 ng/mL.

[C] Do not strip and reprobe PIP Strips, PIP MicroStrips, SphingoStrips or PIP Array membranes. The stability of the individual lipid spots following such treatment has not been tested.

References

1. Curr Opin Cell Biol 13, 146 (2001); **2.** Nat Cell Biol 3, E179 (2001); **3.** Science 292, 2041 (2001); **4.** Science 294, 1881 (2001); **5.** J Cell Biol 153, 429 (2001); **6.** J Biol Chem 275, 288 (2000); **7.** Chem Biol 6, 221 (1999); **8.** J Biol Chem 276, 24985 (2001); **9.** Biochem J 351, 19 (2000); **10.** Biochem J 342, 7 (1999).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
P23748	PIP Array™ membranes *set of 2*	1 set
P23749	PIP Array™ membranes *set of 5*	1 set
P23752	PIP MicroStrips™ membranes *set of 10*	1 set
P23751	PIP Strips™ membranes *set of 10*	1 set
P23750	PIP Strips™ membranes *set of 2*	1 set
S23753	SphingoStrips™ membranes *set of 10*	1 set

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