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Technical Data Sheet

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

Protocol for PIP Strip™ membrane-type Products

For use with product numbers: P-6001, P-6100, P-M600, P-6002, P-6003, S-6000, and S-6001

Procedure (optimized for Echelon's PI(4,5)P_2 Grip™, G-4501)

1. Block: Cover the membrane with 5 to 10 mL of blocking buffer (PBS-T (0.1% v/v Tween-20) + 3% BSA) and gently agitate for one hour at room temperature (RT) or overnight at 4°C.

{Note: during all incubation and wash steps, make sure the membrane stays wet and never dries. We recommend gentle agitation during all incubation and wash steps. We have found that protein-lipid binding can be affected by the blocking buffer, we recommend trying different buffers for each protein of interest, see below for suggested buffers.}

2. Add Protein of interest: Discard blocking buffer and add 0.5 µg/mL PI(4,5)P_2 Grip™ (catalog G-4501) protein in 5 mL PBS-T 3% BSA, or enough to cover the membrane. Incubate for 1 hr at RT with gentle agitation.

{Note: 0.5 ug/mL is given as a starting concentration. The end user must optimize protein concentration for each protein of interest. If high background is experienced or a protein interacts with multiple lipids instead of showing the expected specificity, we recommend decreasing the amount of protein used or running the protein in a different blocking buffer. We do not recommend incubating the protein overnight at 4°C. This may degrade the protein and cause a decrease in activity. We do not recommend using cell lysate, only purified protein or antibodies.}

3. Wash: Discard the protein solution and wash with 5 mL PBS-T three times with gentle agitation for five to ten minutes each.

4. Anti-GST antibody: Discard wash solution and add anti-GST monoclonal antibody (Sigma # G1160) diluted 1:2,000 in PBS-T 3% BSA blocking solution and incubate for 1 hr at RT with gentle agitation.

{Note: The primary and secondary antibodies listed are used routinely at Echelon. Other similar antibodies are likely to work effectively in protein-lipid overlay assays. We recommend including "no primary antibody" and "no secondary antibody" control experiments.}

5. Wash: As in step 3

6. Anti-mouse HRP antibody: Discard wash solution and add anti-mouse IgG-HRP (Sigma # A-5278 or A-9917) diluted 1:2,000 in PBS-T 3% BSA blocking solution and incubate for 1 hr at RT with gentle agitation.

{Note: We recommend including a secondary and detector control. Test the secondary HRP antibody with the detector by spotting 1 µL of HRP conjugate on the strip before running the protein lipid overlay assay. The spot will show up strongly with the detector, indicating that both are working.}

7. Wash: As in step 3

8. Detect: Discard wash solution and detect the bound protein by detection methods of choice e.g., Echelon's K-TMBP, TMB Precipitating NeA Blue from Clinical Science Products, Inc or similar, infrared detection using the Li-Cor system, *Chemiluminescent or ECL™ detection from KPL or similar.

K-TMBP: After discarding the final wash, add 1 to 2 mL TMB Precipitating (catalog K-TMBP) per membrane with gentle agitation. Protein-lipid interaction will develop within 1 to 10 minutes with the spots turning purple. Stop the reaction by discarding K-TMBP and adding 2 mL dH_2O per membrane.

{*Chemiluminescent or ECL detection may produce variable results depending on the kind of ECL used, exposure times, volume, film, and development analysis software. ECL is prone to loosing activity close to and after the expiration date labeled on the bottle.}

{Note: Please see the Echelon Frequently asked Questions Troubleshooting Guide for additional information and recommendations. We **do not** recommend stripping and re-probing the membrane strips or arrays using Western/protein blot protocols. The stability of the individual lipid spots following such treatment has not been confirmed.}

Suggested Buffers for Optimization

TBS Wash Solution 10 mM Tris 150 mM NaCl pH 8.0. For TBS-T Add 0.1% (v/v) Tween-20	**PBS Wash Solution** Dissolve PBS Tablet (Sigma P4417) in 200 mL H_2O. For PBS-T Add 0.1% (v/v) Tween-20	**TBS or PBS + 3% BSA Blocking Solution** Add 3 g fatty acid free BSA (Sigma A7030) to 100 mL TBS or PBS	**TBS or PBS + 1% milk Blocking Solution** Add 1 g non-fat dry milk to 100 mL TBS or PBS	**TBS or PBS +0.1% ovalbumin Blocking Solution** Add 0.1 g ovalbumin (Sigma A5253) to 100 mL TBS or PBS

Echelon's Positive Controls

PIP Grip Proteins	Name	Catalog	Specificity	Optimized Buffer	Concentration
Multi-PIP Grip	LL5α	G-9901	Multiple PIPs and Lipids	PBS-T 3% BSA or TBS-T 3% BSA	0.5 µg/mL
PI(3)P Grip	p40PX	G-0302	PI(3)P	PBS-T 3% BSA	0.5 µg/mL
PI(4)P Grip	SidC_3C	G-0402	PI(4)P	PBS-T 3% BSA	0.5 µg/mL
$PI(4,5)P_2$ Grip	PLCδ1	G-4501	$PI(4,5)P_2$	PBS-T 3% BSA or PBS 1% Milk	0.5 µg/mL
$PI(3,4,5)P_3$ Grip	Grp1	G-3901	$PI(3,4,5)P_3$	PBS-T 3% BSA or PBS 1% Milk	0.1 µg/mL
Sphingomyelin Grip	Equinatoxin	G-SM01	Sphingomyelin	TBS 3% BSA	1 µg/mL

Antibodies	Catalog	Specificity	Optimal Buffer	Concentration
Anti-PI(4)P IgM	Z-P004	PI(4)P	PBS-T 3% BSA	0.5 µg/mL
Anti-$PI(3,4)P_2$ IgG	Z-P034b	$PI(3,4)P_2$	PBS-T 3% BSA	0.5 µg/mL
Anti-$PI(3,4,5)P_3$ IgG	Z-P345b	$PI(3,4,5)P_3$	PBS-T 3% BSA	0.5 µg/mL
Anti-PIP_n IgG	Z-P999	Multiple PIPs	PBS-T only, Do not pre-block membrane	1 µg/mL

Statement, Notes, and Additional Help

The binding pattern obtained with Echelon Strip products can be different compared to binding interactions determined by other methods and non-Echelon membrane-type products. For example, Yu et al. writes that the compared to surface plasmon resonance analysis, lipid overlay experiments are sensitive but that "caution must be exercised in interpreting its results"(1). Further, results at Echelon indicate that the binding pattern of certain PH-domain containing proteins is altered by the use of different protein concentrations and different blocking and washing buffers. Therefore we provide the preceding protocol as a guide, and strongly encourage researchers to consult the scientific literature and conduct optimization experiments in order to establish the most favorable procedures for their protein of interest. A few references are provided below for your convenience.

In addition to protein-lipid overlay experiments, Echelon recommends researchers use alternative methods to fully characterize the lipid binding preference of a particular protein. In addition to membrane-type products, Echelon has a number of innovative products useful for determining protein-lipid interactions. These products include stabilized liposomes (PolyPIPosomes™ e.g. Y-P039, ref(2)), PIP Beads™ (e.g. P-B00S), and PIP-Plates™ (e.g. H-6300). Please contact our technical service representatives by email at http://www.echelon-inc.com; or by phone, toll-free 866-588-0455, with any questions or to provide feedback and suggestions.

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