# Echelon Biosciences Inc.

## FAQ for Lipid Strip and Lipid Array Products

For use with product numbers: P-6001, P-6100, P-6002, P-6003, S-6000, S-6001, P-6111, and P-P100

**Statement, Notes, and Additional Help**

We recommend all Protein lipid overlay assays should start with the example "Protocol for Lipid Strip and Lipid Array Products" using PBS-T (0.1% v/v Tween 20) 3% BSA blocking buffer with a protein starting concentration of 1-2 µg/mL before testing other buffers. We have found this buffer and protein concentration to work well with many of the proteins and antibodies that we have tested. We have also found that protein-lipid binding can be affected by the blocking buffer, and recommend trying different buffers for each protein of interest. See example protocol for suggested buffers.

**High Background - Resulting in overall membrane background, or a black membrane.**

Concentrations of either primary and/or secondary antibody are too high: Using high concentrations of detector proteins and antibodies can cause high background and increased binding to non-specific lipids. Incubating the protein of interest with the secondary antibodies in one step can also cause high background. Each protein, primary, and secondary antibodies should have their own incubation step followed by a wash step.

Cross reactivity between blocking agent and primary antibody: Can be eliminated with the addition of detergent (Tween-20, 0.1% v/v or higher) to the washing buffer and/or incubation buffers. If background persists, changing the blocking buffer is recommended.

Too few wash steps: Washing the membrane between incubation steps is essential in reduction of background signal caused from non-specific binding of the primary and/or secondary antibodies to the membrane. Increasing the number and/or the length of each wash step can help to reduce the background. We suggest many short washing steps over a few long ones such as 3 or more washes for 5 minutes each. A wash step between blocking the membrane and the addition of the protein of interest is unnecessary. Make sure the wash buffer volume is more than the previous protein/antibody incubation volume.

Incubation times of either primary or secondary antibodies are too long: We recommend one hour incubation times at room temperature for each protein and antibody. Only the blocking step can be safely done overnight at 4°C without causing high background. The longer the incubation time of the primary and/or secondary antibodies, the greater the chance of non-specific binding. If long incubation times are necessary to increase protein binding, you might try raising the incubation temperature (eg. to 37 °C) instead.

Membrane allowed to dry between or during incubation steps: Care should be taken, between and/or during incubation steps, to keep the membrane from drying out.

**Little or No Signal - Resulting in little or no signal over membrane background, or a white membrane.**

Primary binding protein has lost activity: Primary binding proteins have unknown stability and have a high probability of loosing activity over time; even with subaliquoting, low freeze-thaw cycles, and -80C storage. Primary binding proteins loosing activity is the most common problem our customers experience. EBI carries several positive control proteins to validate the assay performance in your lab. A positive control protein can help identify if the protein you are using has lost activity towards its specified lipid. See example protocol for list of control proteins and antibodies. Lipid Strips and Lipid Arrays are extremely stable.

Detergent is too harsh: SDS, Nonidet P-40, Triton X-100, and Tween-20 can disrupt binding between proteins and lipids. Removal of detergent from incubation and wash steps can increase protein binding to its target, but may also cause increased non-specific binding to the membrane or a higher overall background. If high background is a concern remove detergent from blocking buffer, but keep in wash buffer.

Inhibition of Secondary antibody HRP conjugate: HRP labeled antibodies should not be used in the presence of sodium azide or hemoglobin. Your protein of interest and HRP conjugated antibody can be spotted directly onto the membrane before blocking as a positive control.

Lipid target is not recognized by lipid recognition protein: Protein-lipid binding can be affected by the blocking buffer, we recommend trying different buffers for each protein of interest. We also recommend researchers use alternative methods to fully characterize the lipid binding preference of a particular protein. Some proteins of interested do not work with these membrane type products.

Detection reagents are expired: ECL and K-TMBP lose activity over time. Verify the expiration date. Also see note under 'Inhibition of secondary antibody'.

**Binding specificity: Non-specific or different than what was expected.**

Detergent is too harsh: SDS, Nonidet P-40, and Triton X-100 disrupt binding between proteins. Tween-20 is the most commonly used and recommended detergent for washing and incubation solutions. Removal of the detergent from incubation steps has been known to change binding specificities of some proteins.



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Incubation time with ECL or Exposure time of film with membrane: The amount of time that the film is exposed to the membrane can increase or decrease the amount of signal that the film is able to detect, or how specific your protein apears. Try longer incubation times with the film if little or no signal is immediately detected. This length of time is determined by how strongly the primary and/or secondary antibody binds and, therefore, may need to be optimized for your specific protein. Example shown below.

Note: Spotting the dry membrane with 1 µL of protein of interest and 2° antibody before the blocking step are good controls for HRP conjugate binding to your protein, the HRP tag, and the detection reagent (ECL or K-TMBP) you use.

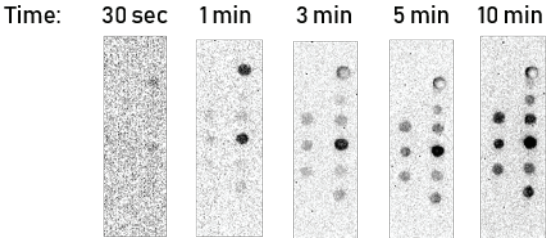


Figure 1:
Multi-PIP Grip (catalog G-9901) was used on PIP Strips (catalog P-6001) at 0.5 µg/mL in TBS-T 3% BSA. Developed with chemiluminescent solution ECL provided by KPL (catalog 54-71-00) for increasing time intervals until the membrane was fully developed at 10 minutes. Note the "halo effect" or "coffee ring" on the top right spot. This occasionally occurs with ECL development when the protein-lipid binding signal is oversaturated. Halos occur more often with less specific proteins as seen here with the Multi PIP Grip

Blocking Buffer: Use of alternative blocking solutions can result in lowered background, increased specificity, and changes in PIP binding patterns. If you use BSA, use the fatty acid free variety. The addition of 1% non-fat dry milk, or 0.1% Ovalbumin to a buffer has been showed to decrease non-specific binding. TBS-T 1% non-fat dry milk in TBS-T is a satisfactory replacement for TBS-T +3% BSA blocking solution. Switching from PBS or TBS based buffers can also affect binding causing an increase or decrease in specificity. Example shown below.

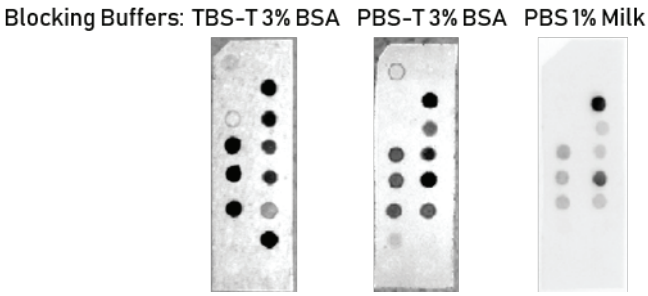


Figure 2:
Multi-PIP Grip (catalog G-9901) was used on PIP Strips (catalog P-6001) at 0.5 µg/mL in three different blocking buffers. Strips were developed with TMB Precipitating solution (catalog K-TMBP).



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