

PRODUCT INFORMATION



Cayman LipiDOT Strips™ - PIPs Plus

Item No. 38924

Storage: 2-8°C
Stability: ≥2 years

Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Description

Cayman LipiDOT Strips™ - PIPs Plus is a nitrocellulose membrane-based screening tool for the identification of protein-lipid interactions and assessment of a protein's relative lipid-binding specificity and affinity. It contains several types of lipids, such as phospholipids, including a variety of phosphatidylinositol phosphate (PIP) species, lysophospholipids, and cardiolipin, as well as cholesterol and sphingolipids. These lipids interact with a variety of proteins to regulate signaling events, nutrient and energy metabolism, lipid transport, and cell adhesion, among other processes.¹

Cayman LipiDOT Strips™ - PIPs Plus contains 22 biologically relevant lipids and two blank (blue) spots for adding a positive control (protein) or using as a negative control and/or additional lipids in the protein-lipid overlay (PLO) assay. The lipids and controls are spotted on a 3.175 cm x 6 cm supported nitrocellulose membrane in a 3 x 8 grid. This product can be used with Cayman LipiDOT Strips™ - Developing Chamber (Item No. 39380) for the blocking, incubation, and washing steps.

Reagents needed for detecting control protein-lipid interaction:

| Reagent | Product Name | Item Number | Description |
|--------------------|---|-------------|--|
| Control Protein | PtdIns-(4,5)-P ₂ (PI(4,5)P ₂) Binding Protein (GST-tagged) | 10009815 | 2 µg/ml in blocking buffer |
| Primary Antibody | GST-tag Polyclonal Antibody | 10010013 | 1:1,500 dilution in blocking buffer |
| Secondary Antibody | Goat Anti-Rabbit IgG HRP | 10004301 | 1:4,000 dilution in blocking buffer |
| Wash Buffer | | | PBS-T (1X PBS, pH 7.2, with 0.1% (v/v) Tween 20) |
| Blocking Buffer | | | PBS-T + 3% fatty acid-free BSA |

Protocol

Optimized procedure for Cayman LipiDOT Strips™ - PIPs Plus:

- Load additional lipid of interest to the membrane (optional):** Spot 1 µl of an additional lipid at 100 µM in a solution, such as chloroform:methanol:water (20:9:1), onto a blue spot on the membrane. Allow the membrane to dry completely in a fume hood.
- Load a positive control protein to the membrane (optional):** Spot 0.1-2.0 µg of the protein (1-2 µl), prepared in a suitable aqueous buffer, onto a blue spot on the membrane. Allow the membrane to dry completely in a fume hood.
- Load a negative control solution to the membrane (optional):** Spot 1-2 µl of solvent or aqueous buffer alone onto a blue spot on the membrane as a negative control. Allow the membrane to dry completely in a fume hood.
- Block:** Cover the Cayman LipiDOT Strips™ membrane in 5-12 ml of freshly prepared blocking buffer overnight at 4°C with gentle agitation.

WARNING
THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFETY DATA
This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

WARRANTY AND LIMITATION OF REMEDY
Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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5. **Add the protein of interest:** Discard the blocking buffer and cover the membrane in 5-12 ml of protein solution, at a concentration of 0.5-4 $\mu\text{g/ml}$, in blocking buffer. Incubate at room temperature for 1 hour with gentle agitation.
6. **Wash:** Discard the protein solution and wash with at least 10 ml of wash buffer 5-6 times, for 10 minutes each, at room temperature with gentle agitation.
7. **Incubate with a primary antibody:** Discard wash buffer and add an appropriate primary antibody prepared in blocking buffer. Incubate at room temperature for 1 hour with gentle agitation.
8. **Wash:** As in Step 6. If a secondary antibody is not needed for detection, skip to Step 11 after this wash.
9. **Incubate with a secondary antibody:** Discard wash buffer and add an appropriate secondary antibody prepared in blocking buffer. Incubate at room temperature for 1 hour with gentle agitation.
10. **Wash:** As in Step 6.
11. **Detect:** Discard wash buffer and detect the bound protein using the detection method of choice.

Suggested Buffers for Optimization:

| | |
|------------------|--|
| Wash Buffers | PBS (pH 7.2) |
| | PBS-T (pH 7.2): 1X PBS, pH 7.2, with 0.1% (v/v) Tween 20 |
| | TBS (pH 7.2): 8 g/L sodium chloride, 0.2 g/L potassium chloride, and 12.1 g/L Tris, pH adjusted |
| | TBS-T (pH 7.2): TBS, pH 7.2, with 0.1% (v/v) Tween 20 |
| Blocking Buffers | TBS or PBS (pH 7.2) + 3% (w/v) fatty acid-free BSA |
| | PBS-T (pH 7.2) + 3% (w/v) fatty acid-free BSA |
| | TBS or PBS (pH 7.2) + 1% (w/v) non-fat dry milk |
| | TBS or PBS (pH 7.2) + 0.1% (w/v) ovalbumin |

Important Notes:

- The binding of certain proteins may be affected by wash buffer or blocking buffer. The use of different solutions for the protein of interest is recommended in case the optimized protocol does not generate ideal results.
- It is important that the Cayman LipiDOT Strips™ membrane stays wet during all incubation and wash steps. The membrane should be completely submerged in wash/blocking buffer. Use more buffer as needed. Gentle agitation is recommended for all incubation and wash steps.
- The use of freshly prepared wash buffer and blocking buffer is recommended. The blocking buffer should be used within 72 hours of preparation and kept at 4°C when not in use.
- The Cayman LipiDOT Strips™ membrane is designed for purified protein or antibodies only. It is not recommended for cell or tissue lysates.
- Stripping and re-probing the Cayman LipiDOT Strips™ membrane is not recommended.

