# Technical Data Sheet

## Avanti Inositol Snoopers™

### Introduction:

Understanding the molecular mechanism behind cellular processes often requires investigating numerous cellular interactions[i]. In this regard, many methods have been developed for assaying and identifying unique protein-protein and protein-DNA interactions[i]. However, one of the most abundant classes of molecules, lipids, has been widely excluded from such assessments suggesting a need for a screening method that allows for the rapid determination of protein-lipid interactions[i]. Avanti Inositol Snoopers consist of 13 different lipid species spotted individually on a solid support. Each spot contains 1 $\mu$g of the highest quality pure lipid and is ideal for investigating lipid-protein interactions.

| | | | | |
|---|---|---|---|---|
| Ref. | 18:1 PI(3,5)$P_2$ | 18:1 PI(4,5)$P_2$ | 18:1 PI(3,4,5)$P_3$ | DMPC |
| 16:0 PI | 18:1 PI | 18:1 PI(3)P | 18:1 PI(5)P | 18:1 PI(3,4)$P_2$ |
| Ref. | Liver PI | Soy PI | Brain PI(4)P | Brain PI(4,5)$P_2$ |

**Figure 1:** Avanti Inositol Snooper™

### Contents:

Five or ten individually packaged Avanti Snoopers are provided. Each ~3 cm x 6 cm snooper is packaged under argon and contains 1 $\mu$g of 13 lipid species. See Figure 1 for the layout of the immobilized lipid species on the Inositol Snooper. For convenience, this template is also found on each individual snooper package.

### Storage:

Avanti Snoopers should be stored as packaged at 4°C.

### Stability:

Avanti Snoopers are stable for 6 months when stored as packaged at 4°C.

### Experimental Protocol:

Materials Needed but Not Provided

- Washing Solution: Tris-Buffered Saline (0.8% NaCl, 20 mM Tris-HCL pH 7.4)
- Blocking Solution: 3% fatty acid free bovine serum albumin (BSA) in Tris-Buffered Saline

| Avanti No. | Description | No. of Strips |
|---|---|---|
| 330500 | Inositol Snoopers™ | Packs of 5 & 10 available |

- Protein/Antibody Diluent: 1% fatty acid free bovine serum albumin (BSA) in Tris-Buffered Saline (TBS)

### General Procedure

- Block: Cover the membrane with ~10 mL of TBS + 3% fatty acid free BSA and incubate for one hour at room temperature with gentle shaking to ensure membrane is completely wetted.*
- Incubate the Snooper with Protein or Antibody of Interest: Remove blocking solution and add desired protein or antibody to the membrane in TBS + 1% fatty acid free BSA for one hour at room temperature with gentle shaking. **
- Wash: Remove protein or antibody solution and wash three times with ~10 mL of TBS for ten minutes with gentle shaking.***
- Secondary Incubation: Following removal of the final wash solution, add a detector or secondary antibody to the membrane in TBS + 3% BSA for one hour at room temperature with gentle shaking.**
- Wash: Repeat wash procedure above to remove any unbound detector or secondary antibody.
- Detection: Following removal of the final wash solution, detect the bound protein or antibody using a method of choice (i.e., colormetric, chemiluminescence, etc.)

Notes:

In general, it is recommended to approach optimization and troubleshooting as you would with Western blots.

* Ensure that the membrane is completely wetted while blocking, and do not allow the membrane to dry between incubations or washes.

** It is essential to optimize the incubation time and concentration of the protein or antibody of interest, as well as the detector or secondary antibody, in order to reduce background resulting from non-specific binding.

*** It may be necessary to optimize wash conditions by increasing the number and/or length

of washes in order to reduce background signal from non-specific interactions.

Tips:

Wash buffer and diluents containing detergent are not recommended for use with this product, as the stability of individual lipid spots may be compromised.

Avanti Snoopers should not be stripped and re-probed. No data is available concerning the fidelity of lipid species following such treatment.

**Application:**

Avanti Snoopers have been used as a method to determine/confirm the specificity of Avanti's WR304 antibody, which recognizes PIP and $PIP_2$.

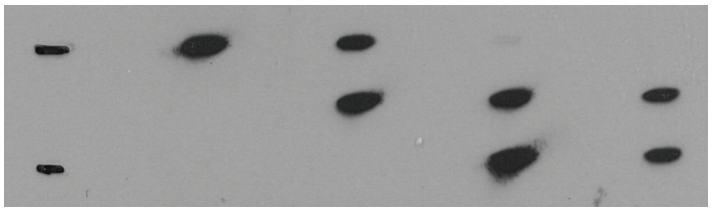

**Figure 2:** Inositol Snoopers were blocked with 3% BSA (fatty acid free) in TBS (0.8% NaCl, 20 mM Tris-HCL pH 7.4) containing 1 mM EDTA. The blocked membranes were then probed with WR304 (1.0 μg/mL) in TBS containing 1% BSA and 1 mM EDTA for 1 h at RT. After washing the membranes 3 times with TBS containing 1 mM EDTA the bound antibody was detected with goat antimouse IgM conjugated to horseradish peroxidase (HRP) (Southern Biotech) at a 1:10,000 dilution in TBS containing 1% BSA and 1 mM EDTA for 1 h at RT. After washing, bound HRP was visualized on an X-ray film with an enhanced chemiluminescence substrate (Pierce).

**Reference:**

[i]Gallego, O., M.J. Betts, J. Gvozdenovic-Jeremic, K. Maeda, C. Matetzki, C. Aguilar-Gurrieri, P. Beltran-Alvarez, S. Bonn, C. Fernandez-Tornero, L.J. Jensen, M. Kuhn, J. Trott, V. Rybin, C.W. Muller, P. Bork, M. Kaksonen, R.B. Russell, and A.C. Gavin. (2010). A systematic screen for protein-lipid interactions in Saccharomyces cerevisiae. *Mol Syst Biol* 6:430.
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