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# Membrane Tube Assay

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1 Works for me



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[dx.doi.org/10.17504/protocols.io.ewov1n3dpgr2/v1](https://dx.doi.org/10.17504/protocols.io.ewov1n3dpgr2/v1) Liv Jensen

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## ABSTRACT

This protocol details about the Membrane Tube Assay.

## ATTACHMENTS

[416-897.pdf](#)

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## PROTOCOL CITATION

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## KEYWORDS

Membrane Tube Assay, Imaging buffer, Biotinylated GUVs, ASAPCRN

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#### OWNERSHIP HISTORY

May 03, 2022  maria.s

May 25, 2022  Liv Jensen

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## MATERIALS TEXT

### Materials:

- Biotinylated GUVs (0.001% mol fraction DSPE-PEG(2000) Biotin, Avanti Polar Lipids), formed by PVA swelling method.
- Small volume flow cells of the type commonly employed for in vitro single molecule imaging (melted parafilm sandwiched between no. 1.5 coverglass)
- Streptavidin functionalized silica beads,  $\rightarrow$  **1.56  $\mu$ m** diameter (Spherotech)
- Bovine serum albumin (BSA)
- Confocal fluorescence microscope modified with an optical trap.
- Fluorescently labeled protein

### Imaging buffer

( iso- osmotic to GUV swelling solution)

A	B
Tris pH 8.0	20 mM
NaCl	150 mM
TCEP	5 mM
MgCl <sub>2</sub>	2 mM

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## Membrane Tube Assay

1 Passivate flow cell with  **1 mg/mL** BSA in imaging buffer.

2 

Rinse flow cell with 2 flow cell volumes of imaging buffer.

3 

Mix GUVs with fluorescent protein and add to flow cell, allowing GUVs to settle on the bottom surface of the flow cell.

4 

Add  **1 µL** of a 1:1000 dilution of silica beads to flow cell.

5 Trap a bead in the optical trap, bring into contact with a GUV, and retract, forming a membrane tube.

6 

Visualize protein recruitment to membrane tube with confocal microscopy.