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Membrane Tube Assay

Liv Jensen[1]

[1]Hurley Lab

dx.doi.org/10.17504/protocols.io.ewov1n3dpgr2/v1

Liv Jensen

DISCLAIMER

ABSTRACT

This protocol details about the Membrane Tube Assay.

ATTACHMENTS

416-897.pdf

DOI

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PROTOCOL CITATION

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KEYWORDS

Membrane Tube Assay, Imaging buffer, Biotinylated GUVs, ASAPCRN

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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, **1.56 µm** 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
MgCl2	2 mM

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Membrane Tube Assay

1 Passivate flow cell with [M]**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.

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