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GUV preparation

GUV preparation and assay

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GUV preparation for membrane tube assay application

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1 GUV Preparation

1.1 Clean the coverglass.

1.2 Coat cleaned coverslips with 100 µL 5% (w/w) polyvinyl alcohol (PVA) with a

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molecular weight of 145,000 (Millipore).

1.3 Place the coated coverslip in a heating incubator at 60 °C to dry the PVA film for 30 min.

1.4 Spread a lipid mixture with a molar composition of 70% DOPC, 20% DOPE, 5% DO-PI(3)P, 5% DOPS, 0.3% Atto647N DOPE, 0.01% PEG2000-biotin-DSPE at 1 mg/ml uniformly onto the PVA film.

1.5 Put the lipid-coated coverslip under vacuum overnight to evaporate the solvent.

1.6 Use 100 µL 320 mOsm sucrose solution for swelling for 1 h at room temperature

1.7 Harvest the GUVs and use them with 12 h.

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