

Giant Unilamellar Vesicles (GUVs) Preparation by Electroformation Method

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Abstract

Preparation of giant unilamellar vesicles (GUVs) by electroformation method has been detailed. 10 to 100 micrometer size GUVs obtained after the process.

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Materials



Sucrose 57903 by Sigma Aldrich



Chloroform 366919-1L by Sigma



1,2-Dioleoyl-sn-glycero-3-phosphocholine
P6354 by Sigma Aldrich



Texas Red™ 1,2-Dihexadecanoyl-sn-
Glycero-3-Phosphoethanolamine,
Triethylammonium Salt T1395MP by Thermo
Fisher Scientific

Protocol

Preparation of phospholipid stock solution

Step 1.

Weigh phospholipid of interest, dissolve the powder in chloroform at 10 mM final concentration.

Step 2.

For fluorescent GUVs, add fluorescent phospholipid Texas-Red-DHPE (1 mM stock) to reach the final molar ratio of 1:500.

Electroformation by using Nanion Vesicle Prep Pro

Step 3.

Identify the conductive sides of the ITO-slides with a multimeter.

Electroformation by using Nanion Vesicle Prep Pro

Step 4.

Use a 5 µl Hamilton syringe to spread 10 µl of phospholipid stock solution on one of the ITO glass slide.

Electroformation by using Nanion Vesicle Prep Pro

Step 5.

Leave the ITO coating side upwards into a vacuum dessicator for 15 min.

Electroformation by using Nanion Vesicle Prep Pro

Step 6.

Prepare 195 mM sucrose solution, dissolve it in Hepes Buffer 5 mM pH 7.4, vortex until sucrose is dissolved.

Electroformation by using Nanion Vesicle Prep Pro

Step 7.

Spread a thin layer of vacuum grease evenly on the O-ring that you use.

Electroformation by using Nanion Vesicle Prep Pro

Step 8.

After the ITO-slides are dried, take the top part off from the Nanion Vesicle Prep Pro chamber by removing the screws.

Electroformation by using Nanion Vesicle Prep Pro

Step 9.

Use tweezers to place the ITO-slide lipid film point upwards in the Nanion Vesicle Prep Pro.

Electroformation by using Nanion Vesicle Prep Pro

Step 10.

Place an O-ring around the dried lipid film, add 280 µl of 195 mM sucrose solution into the O-ring.

Electroformation by using Nanion Vesicle Prep Pro

Step 11.

Place a second ITO-slide on top of the O-ring with the conductive side facing downwards using tweezers. Note that, the right end of the top slide has to touch properly to the electrode on the right-side.

Electroformation by using Nanion Vesicle Prep Pro

Step 12.

Place the top part of the chamber back on and screw it carefully.

Electroformation by using Nanion Vesicle Prep Pro

Step 13.

Turn-on the Nanion VPP, select the appropriate protocol and initiate the formation process.

TEMPERATURE

37 °C Additional info:

DURATION

00:05:00 Additional info: Frequency 5 Hz, applied voltage from 0 to 3 V

DURATION

02:00:00 Additional info: Frequency 5 Hz, applied voltage 3 V

DURATION

00:05:00 Additional info: Frequency 5 Hz, applied voltage from 3V to 0

Electroformation by using Nanion Vesicle Prep Pro

Step 14.

After the protocol ends remove the screws and take off the top part of the chamber. Use the tweezers to remove the top ITO-slide.

Electroformation by using Nanion Vesicle Prep Pro

Step 15.

Cut a pipette tip and collect the sample from the ITO-slide surface slowly.

Fluorecence Microscopy

Step 16.

Withdraw a 5 µl aliquot of the electroformation product and inspect the GUVs under an epifluorescence microscope.

Warnings

Lipid stock solutions should be prepared in the **FUME HOOD**, on ice!