Preparation of Proteoliposomes

Zehra Kahveci

Abstract
Detergent mediated reconstitution of membrane proteins.

Citation: Zehra Kahveci Preparation of Proteoliposomes. **protocols.io**
dx.doi.org/10.17504/protocols.io.rvwd67e
Published: 23 Jul 2018

Materials

Poly(ethylene glycol) octyl ether 40530 by Sigma Aldrich

Bio-Beads™ SM-2 Resin 1523920 by BioRad Sciences

Protocol

Liposomes preparation

Step 1.

PROTOCOL

. Giant Unilamellar Vesicles (GUVs)

Preparation by Electroformation Method

CONTACT: Zehra Kahveci

Preparation of phospholipid stock solution

Step 1.1.
Weigh phospholipid of interest, dissolve the powder in chloroform at 10 mM final concentration.

Step 1.2.
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 1.3.
Identify the conductive sides of the ITO-slides with a multimeter.

Electroformation by using Nanion Vesicle Prep Pro

Step 1.4.

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 1.5.

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

Electroformation by using Nanion Vesicle Prep Pro

Step 1.6.

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 1.7.

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

Electroformation by using Nanion Vesicle Prep Pro

Step 1.8.

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 1.9.

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 1.10.

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

Electroformation by using Nanion Vesicle Prep Pro

Step 1.11.

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 1.12.

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

Electroformation by using Nanion Vesicle Prep Pro

Step 1.13.

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 1.14.

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 1.15.

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

Fluorecence Microscopy

Step 1.16.

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

Proteoliposomes-DAY1

Step 2.

Prepare 2 eppendorfs containing 100 µL of 195 mM sucrose solution (freshly made in Hepes buffer 5mM pH 7.4) and add 100 µL of electroformation product into each eppendorf.

Proteoliposomes-DAY1

Step 3.

Shake the Eppendorfs gently upside-down.

Proteoliposomes-DAY1

Step 4.

Dilute the membrane transporter (5mg/mL) in Poly(ethylene glycol) octyl ether (**o-poe**), 1:1 (v:v), total volume of 1µL.

Proteoliposomes-DAY1

Step 5.

Add 1µL of membrane transporter:o-poe 1:1 (v:v) into Eppendorf 1 **(SAMPLE)**

Proteoliposomes-DAY1

Step 6.

Add 1µL of o-poe into Eppendorf 2 **(BLANK)**

Proteoliposomes-DAY1

Step 7.

Shake the SAMPLE and BLANK gently upside-down.

Proteoliposomes-DAY1

Step 8.

Place both tubes on to a shaker, shake them at 600 rpm.

TEMPERATURE

24 °C Additional info:

DURATION

01:00:00 Additional info:

Proteoliposomes-DAY1

Step 9.

Add 2.5 mg Biobeads into both tubes.

Proteoliposomes-DAY1

Step 10.

Shake the tubes upside-down, place them on to a shaker and shake them at 600 rpm.

TEMPERATURE

24 °C Additional info:

DURATION

01:00:00 Additional info:

Proteoliposomes-DAY1

Step 11.

Store the SAMPLE and BLANK in the fridge at 4°C, overnight.

Proteoliposomes-DAY 2

Step 12.

Cut a pipette tip, collect the SAMPLE and the BLANK from each each eppendorf and put the SAMPLE and BLANK into new eppendorfs.

Proteoliposomes-DAY 2

Step 13.

The SAMPLE and the BLANK can be used in the following 4-5 days. (Store in the fridge).