



AUG 25, 2023

Liposome preparation

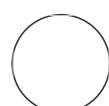
Xinbo

Wang^{1,2}, Pietro De Camilli^{1,2}

¹1. Departments of Neuroscience and of Cell Biology, Howard Hughes Medical Institute, Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, Connecticut 06510, USA;

²2. Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

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ABSTRACT

This protocol details methods for the preparation of 100% PS liposome and GC/PS lipid nanotubes used for LRRK2 binding, tubulation assays.

ATTACHMENTS

[iuucbv9p.docx](#)

DOI:

dx.doi.org/10.17504/protocols.io.6qpvr612ovmk/v1

Protocol Citation: Xinbo Wang, Pietro De Camilli 2023. Liposome preparation. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.6qpvr612ovmk/v1>

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Protocol status: Working
We use this protocol and it's working

Created: Aug 19, 2022

Last Modified: Aug 25, 2023

PROTOCOL integer ID:
68882

Keywords: Liposome,
LRRK2, Lipid nanotube

MATERIALS

⊗ Brain PS Avanti Polar Lipids,
Inc. Catalog # 840032

⊗ Galactosylceramide (GC) Avanti Polar Lipids,
Inc. Catalog #860546P

⊗ Rhod-PE Avanti Polar Lipids,
Inc. Catalog #810150

⊗ Cy5-PE Avanti Polar Lipids,
Inc. Catalog #810345



Solutions to prepare:

Liposome buffer:

A	B
HEPES (7.4)	20 mM
KCl	100 mM
TCEP	0.5 mM


Liposome preparation

1h 5m




- 1 Dissolve lipid mixtures with chloroform in glass vials in moles percent as follows:
 - **PS liposomes:** 99.5% brain PS:0.5% Rhod-PE.
 - **GC/PS nanotubes:** 39.5% Galactosylceramide:60% brain PS:0.5% Cy5-PE.
- 2 Evaporate chloroform under a stream of nitrogen gas to produce a lipid film on the glass surface.
- 3 Dry the lipid film in a vacuum oven for  01:00:00 . 1h
- 4 Rehydrate the dried lipid films in liposome buffer at a final concentration of  1 mg/mL (~



[M] 1.2 millimolar (mM)).

5 For PS mixtures, form the liposomes by three freeze (liquid N₂)–thaw ( 37 °C water bath) cycles.

5.1 For GC/PS mixtures, form the lipid nanotubes by a brief vortexing instead of freeze-thaw cycles.

6 Remove large aggregates by a brief centrifugation ( 500 x g for  00:05:00) and store in the dark at  4 °C to avoid photooxidation.

5m