

May 31, 2023

GFP-ATG3 GUV Assay

DOI

<https://dx.doi.org/10.17504/protocols.io.yxmvm2d5ng3p/v1>

Imstrong¹

¹UC Berkeley



Imstrong

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.yxmvm2d5ng3p/v1>

Protocol Citation: Imstrong 2023. GFP-ATG3 GUV Assay . protocols.io
<https://dx.doi.org/10.17504/protocols.io.yxmvm2d5ng3p/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: May 31, 2023



Last Modified: May 31, 2024

Protocol Integer ID: 82660

Keywords: ASAPCRN, atg3 guv assay, lc3 lipidation, atg3, guv, assay, gfp, atg3 guv

Funders Acknowledgements:

ASAP

Grant ID: ASAP-000350

Abstract

LC3 lipidation on GUVs

1 GUV Preparation

1.1 Clean the coverglass

1.2 Coat cleaned coverslips with 100 μ L 5% (w/w) polyvinyl alcohol (PVA) with a molecular weight of 145,000 (Millipore). Place the coated coverslip in a heating incubator at 60 °C to dry the PVA film for 30 min.

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

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

1.5 Use 100 μ L 330 mOsm sucrose solution for swelling for 1 h at room temperature

1.6 Harvest the GUVs and use them with 12 h.

2 GUV Assay

2.1 Set up the reaction in an eight-well observation chamber (Lab Tek) at room temperature.

2.2 Coat the chamber with 1 mg/ml β casein for 10 min.

2.3 Wash the coated chamber with reaction buffer (25 mM HEPES at pH 7.4, 150 mM NaCl and 1 mM TCEP).

2.4 Make a 120 μ L reaction mixtures with the proteins and 5 mM ATP and 1 mM MgCl₂. The final concentration of WIPI2d 200 nM, E3 complex is 50 nM, ATG7 is 100 nM, GFP-ATG3 or Mutant is 100 nM, and mCherry-LC3B is 500 nM.

2.5 Add 3 μ L GUVs to initiate the reaction.



2.6 Pick random views for imaging within 5 min.