



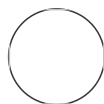
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## LC3-lipidation-assay

DOI

[dx.doi.org/10.17504/protocols.io.kqdg392ypg25/v1](https://dx.doi.org/10.17504/protocols.io.kqdg392ypg25/v1)[Liv Jensen](#)<sup>1</sup><sup>1</sup>University of California, Berkeley

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COMMENTS 0

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## ABSTRACT

Protocol for an in vitro LC3 lipidation assay using purified proteins and synthetic liposomes.

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## MATERIALS TEXT

ATG3, ATG7, WIPI2, ATG12–5-16L1-GFP, LC3B

400nm extruded liposomes (70% DOPC, 20% DOPE, 5% DOPS, 5% PI(3)P)

Reaction buffer: 20mM Tris pH8.0, 150mM NaCl, 1mM MgCl<sub>2</sub>, 1mM TCEP, 1mM ATP

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- 1 Mix 2µM ATG3, 2µM ATG7, 1µM WIPI2, 200nM ATG12–ATG5-ATG16L1-GFP, 5µM LC3B 1:1 with extruded liposomes in reaction buffer.
- 2 Incubate at 37°C
- 3 At 0, 15, 30, 60, and 120 minutes, remove 12µl of reaction mixture and quench by adding 4ul of SDS-PAGE loading buffer and heating at 60°C for 10 minutes.

