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LC3-lipidation-assay

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We use this protocol and it's working

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Abstract

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

Materials

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 MgCl₂, 1mM TCEP, 1mM ATP



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