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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

Troubleshooting

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℃

3 At 0, 15, 30, 60, and 120 minutes, remove 12µl of reaction mixture andquench by adding 4ul of SDS-PAGE loading bufferandheatingat 60℃ for 10 minutes.