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LC3 Lipidation assay for ATG3 Mutants

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Protocol status: Working

We use this protocol and it's working

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Abstract

LC3 lipidation assay on liposomes



- 1 Mix 1 μ M ATG3 WT or mutant, 1 μ M ATG7, 500 nM WIPI2d, 1 μ M ATG12–ATG5–ATG16L1, 5 μ M LC3B and 0.5 mg/mL extruded liposomes in reaction buffer (25mM HEPES pH 7.4, 150 mM NaCl, 1 mM TCEP).
- 2 Incubate at room temperature for 20 minutes.
- 3 Quench with 4X LDS loading buffer. Boil samples for 10 minutes at 60°C. Load and run SDS PAGE gel
- 4 Visualize protein bands with coomassie blue