




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MBP Pulldown Assay of ATG9A Truncations

 Forked from [WIPI2d Coprecipitation Assay](#)Adam Yokom¹, Xuefeng Ren¹¹Team Hurley1 *Works for me* Sharedx.doi.org/10.17504/protocols.io.e6nvwk7xwvmk/v1

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ABSTRACT

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FORK NOTE

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- 1 Equilibrate 30 µl of Amylose resin (New England Biolabs, Ipswich, MA). Add >500mL of wash buffer (25 mM HEPES pH 7.5, 150mM NaCl, 1mM MgCl₂, 1mM TCEP). Slow spin to pellet resin. ~1000rpm for 1 minute should be good. Repeat X3
- 2 Add recombinant MBP protien (~1 uM) and ATG13:ATG101 dimer (~3 uM) to Amylose resin
- 3 Incubate overnight at 4C

- 4 Wash resin 4x with wash buffer (25 mM HEPES pH 7.5, 150mM NaCl, 1mM MgCl₂, 1mM TCEP)
- 5 Elute samples in 50 uL buffer + 50 mM Maltose
- 6 Mix eluted samples with lithium dodecylsulfate (LDS)/BME buffer. Heat at 60C for 5 min and run on SDS/PAGE gel
- 7 Quantify using Fiji ImageJ2