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MBP Pulldown Assay of ATG9A Truncations



Forked from [WIP12d Coprecipitation Assay](#)

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We use this protocol and it's working

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Abstract

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- 1 Equilibrate 30 μ l 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 μ M) and ATG13:ATG101 dimer (~3 μ M) 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 μ L 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