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MBP Pulldown Assay of ATG9A Truncations

Forked from WIPI2d Coprecipitation Assay

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We use this protocol and it's working

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Abstract

MBP Pulldown Assay of ATG9A Truncations

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