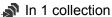


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**ABSTRACT** 

Traditional in vitro pulldown





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## **Pulldown** 1 In a final volume of 30 μL, 20 μg of purified MBP-Rubicon RH domain was mixed with 20 μg of RAB7A in 50 mM HEPES 7.5, 150 mM NaCl, 2 mM MgCl2, 10 mM TCEP buffer. Include a negative control consisting of only soluble Rab7, with no MBP-Rubicon, in order to test your washing efficacy. 2 Incubate samples on rocker at room temperature for 1 H 3 Add 20 uL of 50% v/v amylose resin to each sample 4 Allow to rock for an additional 30 min to bind 5 Collect resin at bottom of tube via a tabletop centrifuge, aspirate off and discard supernatant, and wash with 500 uL of ice cold buffer for three total washes. 6 Elute sample by resuspending beads in 20 uL of buffer + 20 mM maltose for 10 min on shaker. 7 Prepare samples for SDS-PAGE by adding 1x loading buffer, and load onto a 4-12% gel. Run gel at 120 V until dye front reaches the bottom of the gel

8 Stain with Coomassie blue G-250, and destain with water until bands are clearly visible. Image.

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