MAR 07, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.5jyl8pw6dg2w/v1

Protocol Citation: Dan Tudorica 2024. Pulldowns . **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl8pw6dg2w/v1

Protocol status: Working

Created: Nov 09, 2023

Last Modified: Mar 07, 2024

Pulldowns

In 1 collection

Dan Tudorica[1]

[1]University of California, Berkeley

Dan Tudorica
Hurley Lab, QB3, UC Berkeley

ABSTRACT

Traditional in vitro pulldown

PROTOCOL integer ID: 90728

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.