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Immunoprecipitations and Western blots analysis V.1

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Leonardo A Parra-Rivas[1]

[1]University of California, San Diego

Leonardo A Parra-Rivas
University of California, San Diego

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We use this protocol and it's working

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Abstract

Immunoprecipitations and Western blots analysis

Troubleshooting

1 Immunoprecipitations were performed using
1-2 mg of total protein. Samples were incubated overnight with the indicated antibody at 4° C, followed by the addition of 50 µl of protein G-agarose beads (Thermo Scientific, Cat#20397).

2 Immunoprecipitated proteins were recovered by centrifugation at 2,500× rpm for 2 min, washed three times with a buffer containing PBS and 0.15% Triton X-100. The resulting pellets were resuspended in 20 µl of 1X NuPAGE LDS sample buffer (Thermo Scientific #NP007) and
incubated at 95 °C for 10 min. Samples were separated by NuPAGE 4 to 12% Bis-Tris polyacrylamide gels (Thermo Scientific #NP0335BOX), and transferred to a 0.2 µM PVDF membrane (Thermo Scientific #LC2002), using the Mini Blot Module system (Thermo Scientific).

3 PVDF membranes were first fixed with 0.2% PFA 1x PBS per 30 min at room temperature. Then, membranes were washed three times for 10 min in PBS with 0.1% Tween 20 Detergent (TBST) and blocked for 1 h in TBST buffer containing 5% dry milk, and then incubated with the indicated primary
antibody for 1 h in blocking solution, washed three times for 10 min each and incubated with HRP-conjugated secondary antibodies (RRID:AB_2819160), (RRID:AB_2755049).

4 After antibody incubations, membranes were again washed three times with TTBS buffer, and protein bands were visualized using the ChemiDoc Imaging System (BioRad) and quantified with Image Lab software version 6.1 from BioRad (RRID:SCR_014210) http://www.bio-rad.com/en-us/sku/1709690-image-lab-software.