Immunoprecipitation
NDJ V.2
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1. Homogenize brains in lysis buffer with 600ul lysis buffer with motor pestle. See collection of brains protocol for Western blot (page is 27 in NDJ protocol book). For cell culture, see cell culture protocol (page 28).
2. Freeze-thaw 3x (liquid N2 to 37°C). Use gel-slick tubes
3. **For Denaturing IP:** Add 100ul 10% SDS (1% SDS) and heat for 5 minutes 95°C. Then, ice for 5 minutes.
4. Spin samples for 5 minutes @ 4°C 2500rpm
5. Transfer supernatant to new tube.
6. Wash beads 3x with lysis buffer. The beads should be in a 50% slurry in non-denaturing lysis buffer. Protein G Sepharose is universal for IgG mouse and rabbits antibodies according to Amersham. See 10.16.3 in Current Protocols in Molecular Biology for more information. Protein A for IgA and IgE antibodies. Wash enough beads for pre-clearing and for conjugating to antibody. To wash:
   a. Add buffer tap and invert 3x to mix.
   b. Spin Max speed 2 seconds. (Or, 3000xg for 2 minute).
   c. Add back volume of lysis buffer
7. Pre-clear sample using protein G (40ul) for 30 minutes on rugged rotator for 30 minutes @ 4°C.
8. Spin Pre-cleared beads 5 minutes maximum speed 16000xg @ 4°C. Transfer lysates (supernatant) to new tube and keep on ice. Leave a 20ul on beads to avoid transfer of non-specific material.
9. Add antibody for each experimental condition. Put on rugged rotator for overnight 2 hours. Amount of antibody varies with affinity of antibody for antigen and a host of other variables. Starting concentration for antibodies with no working data are suggested below. (Optional: add 10% BSA when done to quench non-specific binding.)

1 to 5 ul	polyclonal antiserum
1ug	affinity purified polyclonal
0.2 to 1ul	ascitic fluid monoclonal
1ug	purified monoclonal
20 to 200ul	culture supernatant monoclonal

10. Add washed beads (see 6) to lysates. Incubate on rugged rotator in microfuge tube 4°C for ON (overnight).
11. Spin 5 sec 16000xg 4°C.
12. Aspirate leaving 20ul on top.
13. Add 1ml ice wash buffer. Use 0.1% (w/v) Triton-X in non-denaturing lysis buffer. Can use tween or forego detergent at all
14. Repeat steps 11-13 more.
15. Wash beads once more in ice-cold PBS.
16. Load beads on gels per Western blot protocol.