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Immunoprecipitation protocol

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Protocol status: Working

We use this protocol and it's working

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Abstract

Immunoprecipitation procedure for brain tissue material



- 1 Add 750ug tissue in 1ml IP buffer and antibody, incubate 4°C 2hrs on tube rotator
- 2 Prepare ProteinA/G beads 30ul/sample. Pool all beads in one tube to perform wash
- 3 Centrifuge 4°C 13000g 30sec, remove supernatant
- 4 Resuspend beads with 80ul PBS/sample. Centrifuge again at 13000g for 30sec.
- 5 Repeat wash 3 times.
- 6 Remove supernatant with fine tip
- 7 Resuspend beads with 30ul PBS/sample
- 8 Add 30ul beads to protein sample
- 9 Incubate 4°C overnight on tube rotator
- 10 Centrifuge 4°C 4000rpm 4min, remove supernatant
- 11 Wash with IP buffer (+ protease inhibitor) 800ul/sample
- 12 Repeat 3 times, total 4 wash
- 13 Remove supernatant with fine tip



- 14 Add 1x loading buffer 25ul and 5ul reducing agent. 75°C 5min
- 15 Centrifuge 4°C 4000rpm 4min, collect supernatant for western blotting