Immunoprecipitation protocol

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ABSTRACT

Immunoprecipitation procedure for brain tissue material

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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

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14 Add 1x loading buffer 25ul and 5ul reducing agent. 75°C 5min

15 Centrifuge 4°C 4000rpm 4min, collect supernatant for western blotting

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