Flag M2 immunoprecipitation and Western Blot in Drosophila

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Abstract

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Protocol

Step 1.

Immunoprecipitation with Flag M2 magnetic beads in *Drosophila*

1. Grow flies expressing transgenic constructs under control of the UAS/GAL4 system at 25°

2. Procedure to prepare protein lysate from different developmental stages in *Drosophila*;

1. Embryo collection

- To retrieve a collection of all embryonic stages, harvest 0-22 hour old embryos in a mesh-basket and wash away the yeast paste with deionized H_2
- Dechorinate the embryos in a 1:1 dilution of Sodium hypochlorite 14% (f.c. 7%) and deionized H_2O for 2-4 minutes, rinse embryos for 1 min in deionized water.
- Transfer the embryos in 100 µl portions to 1.5 ml microtubes and mince with pestle in 200 µl Lysis buffer A (50 mM HEPES pH7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 10% Glycerol, 25 mM NAF, 10 µM ZnCl2), supplemented with 1 tablet Complete Inhibitors (Roche) per 50 ml, 1mM PMSF and 10 mM N-ethyl maleimide (NEM).
- Add 800 µl Lysis buffer A to each embryo-portion and continue grinding.
- Incubate all samples on ice for 30 minutes.
- Spin 14 000 x g for 15 min at 4°C and transfer and pool supernatants to fresh tubes.
- Measure protein concentration.

1. Larvae collection

- Collect wandering third instar *Drosophila* larva and rinse them twice in 1xPBS.
- Add 25 larva per aliquot to 1.5 ml microtubes with 125 µl Lysis buffer A (see above) and grind the larvae with a pestle.
- Add 375 µl fresh Lysis buffer A and continue grinding.
- Incubate all samples on ice for 30 minutes.
- Spin 14 000 x g for 15 min at 4°C and transfer and pool supernatants to fresh tubes.
- Measure protein concentration.

- Adult fly collection
- Collect 0-4 day old adults flies under CO_2 anesthesia, 40 flies per aliquot, in 1.5 ml microtubes.
- Grind the flies in 200 µl Lysis buffer A (see above), add another 200 µl and continue grinding.
- Incubate all samples on ice for 30 minutes.
- Spin 14 000 x g for 15 min at 4°C and transfer and pool supernatants to fresh tubes.
- Measure protein concentration.

3. Take out 60 µl from the lysate, mix with 60 µl 2x Sample buffer and incubate 7 min at 98°C (=INPUT).

4. Wash the Flag M2 magnetic beads (Sigma M8823) 3 times in Lysis Buffer A before usage. Make sure that the beads are completely defrosted before aliquoting the slurry with a cut pipet tip. Collect magnetic beads with a magnetic separator.

5. Set up immunoprecipitations with Flag M2 magnetic beads at 4°C, by mixing 1-2 mg total protein with 30 ml Flag M2 magnetic beads (slurry, equals 15 ml packed beads) in 1.5 ml microtubes.

6. Incubate for 2 hours at 4°C on rotator. Collect beads with magnetic separator.

7. Wash 6 times with 1 ml Lysis buffer A (collect beads with a magnetic separator between each wash).

8. Add 25 ml 2X Sample buffer with max 5% β-Mercaptoethanol to the magnetic beads. Incubate the samples at 98°C for 4 min (alternatively the elution can be performed with 0.1 M Glycine-HCl, pH 3.0).

9. Separate the samples for loading using a magnetic separator and transfer the eluted proteins to fresh tubes with a narrow ended tip.

10. Analyze the samples by separating the immunoprecipitated proteins on SDS-PAGE, followed by Western Blot.

Western Blot in *Drosophila*

1. Separate the eluted immunoprecipitates and protein lysates by SDS-PAGE (use a percentage of acrylamide suitable to detect your protein of interest or a gradient gel).

2. Transfer the separated proteins onto a 0.2 mm PVDF membrane (Merck Millipore) using a semi-dry Trans-Blot Turbo transfer system (Bio-Rad).

3. Block the membranes in 5 % BSA in 1X TBS (50 mM Tris-HCl, pH 7.5, 150 mM NaCl) for 1 hour at room temperature.

4. Incubate with primary antibodies, diluted in 1X TBS with 5 % BSA, overnight at 4°

5. Wash the membranes 5 times for 10 min in TBST (1X TBS with 0.075% Tween-20).

6. Incubate with HRP-linked secondary antibodies (GE Healthcare) for 1 hour at room temperature.

7. Wash the membranes 5 times 10 min in TBST at room temperature, and finally 10 minutes with 1X TBS.

8. Develop the membranes using standard techniques and according to the manufacturers recommendations; (i) SuperSignal™ West Pico Chemiluminescent Substrate (Thermo Scientific), ii) ECL™ Prime Western blotting Detection reagent (GE Healthcare).

9. Visualize by standard autoradiography on film or with a C-Digit Blot Scanner analysis (LI-COR Biosciences).