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**Protocol status:** Working
We use this protocol and it's working

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# SOP – 3-step protein fractionation from fly heads

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ABSTRACT

SOP – 3-step protein fractionation from fly heads

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Aligning Science Across
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## Reagents Needed:

1 Protease and Phosphatase Inhibitors (cOmplete 04 693 124 001, PhosphoSTOP 04 906 837 001)

2 Urea – Fisher Bioreagents #BP169-500

3 SDS – Fisher Bioreagents #BP166-500

4 RIPA Buffer (PierceTM, Thermo Scientific 89900)

5 NP-40 (Nonidet® P 40 Substitute, Sigma, Cat #74385)

6

| | A | B | C |
|---|---|---|---|
| | Stock Solutions | Formula | Storage |
| | TBS | 20 mM Tris Base, 150mM NaCl, pH 7.6 | 4C |
| | 20% SDS | 10g SDS in 50ml of MilliQ | Room Temperature; tends to gel up, in which case you have to heat it up on a hot plate |
| | RIPA Buffer | PierceTM, Thermo Scientific 89900 | 4C |
| | 10X PhosphoStop | 1ml of MilliQ; 1 tablet: PhosphoStop | -20C |
| | 10X cOmplete | 1ml of MilliQ; 1 tablet: cOmpleteMini | -20C |

| | A | B |
|---|---|---|
| | TBS | 800uL TBS, 100uL 10X cOmplete, 100uL 10X PhosphoStop |
| | 5% SDS | 550ul TBS, 100ul 10X cOmplete, 100ul 10X PhosphoStop, 250ul 20% SDS |
| | RIPA | 800uL RIPA Buffer, 100uL 10X cOmplete, 100uL 10X PhosphoStop |
| | 1% NP-40 | 790uL TBS, 100uL 10X cOmplete, 100uL 10X PhosphoStop, 10uL NP-40 |
| | 8M Urea/5% SDS | 300ul TBS, 100ul 10X cOmplete, 100ul 10X PhosphoStop, 250ul 20% SDS |
| | | Add 0.480.4g Urea wait to dissolve then bring to 1ml with TBS |
| | | |

Working solutions (w/ PPIs) Make and use the day of the extraction, keep on ice. Formulation per 1ml.

## Protocol:

7 Collect 20-40 fly heads via Snap Freeze method into a Biomasher tube and transfer tubes to CTRND on dry ice.

8 Turn on Sorvall ultracentrifuge and set temperature to 4degC, place rotor inside and turn on vacuum to allow internal temperature to set.

9 Add 50uL (2.5uL/fly head) of TBS with PPI to heads in Biomasher II tube and homogenize for 1.5 min with a hand-held automatic homogenizer and Biomasher pestles.

10 Briefly spin down in tabletop centrifuge to get the liquid to the bottom of the tube.

11 Sonicate at 35% with 5 one-second pulses for tissue to break down/dissolve. Skip this step for 'non-sonicated' extractions.

12 Clarify by centrifugation in a tabletop centrifuge (2,100g) for 15 seconds.

13 Move entire liquid sample into ThermoScientific 5ml microtube WX und MX (Cat #314352H01) before centrifuging.

14 Centrifuge the supernatant at 100,000 g (29,900 rpm) for 30 min at 4°C using the chilled rotor.

15 Remove and save Supernatant into new labeled collection Eppendorf (TBS-soluble fraction).

16 Wash the pellet by adding 50uL of TBS with PPI and centrifuging at 100,000 g for 15 min at 4°C. Discard the supernatant.

17 Homogenize pellet with 30uL (1.5ul/fly head) of solvent with PPI with hand-held homogenizer.

18 Centrifuge at 100,000 g for 30 min at 4°C and save supernatant into new labeled collection Eppendorf (_-soluble fraction).

19 Wash the pellet by adding solvent 2 with PPI and centrifuging at 100,000 g for 15 min at 4°C. Discard the supernatant.

20 Resuspend pellet in 30ul (1.5ul/fly head) in 8M urea/5% SDS buffer with PPI by sonicating with 3 one-second pulses until it is fully resuspended. Label and save (insoluble fraction)

21 Perform BCA. If strapped on time, samples can wait to be quantified and stored at -80C.