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SOP – 3-step protein fractionation from fly heads

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Protocol status: Working

We use this protocol and it's working

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Abstract

SOP – 3-step protein fractionation from fly heads



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 (Pierce™, Thermo Scientific 89900)
- 5 NP-40 (Nonidet® P 40 Substitute, Sigma, Cat #74385)

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	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	Pierce™, 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



A	B
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.