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SOP – 2-step protein fractionation (Triton) from fly heads

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Protocol status: Working

We use this protocol and it's working

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Abstract

SOP – 2-step protein fractionation (Triton) from fly heads

Materials

Vortex

Tabletop centrifuge

Biomasher II Tube/Pest, Sterile – KIMBLE (Cat #9749625002) – for homogenization.

Biomasher II homogenization pestles

ThermoScientific 1.5ml microtube WX und MX (Cat #314352H01) – For ultracentrifugation.

Pipettes, pipette tips

1.5 mL Eppendorf tubes

Waste bucket and bag

Gloves

Thin marker

Dry ice

Ice

Metal tube rack for ice

Reagents Needed:

- 1 Protease and Phosphatase Inhibitors (cOmplete 04 693 124 001, PhosphoSTOP 04 906 837 001)
- 2 Triton X-100 (Millipore Sigma, Cat# T9284)
- 3 NaF (Sigma-Aldrich, Cat# S7920)

3.1

	A	B	C
	Stock Solutions	Formula	Storage
	TBS	20 mM Tris Base, 150mM NaCl, pH 7.6	4C
	10X PhosphoStop	1ml of MilliQ; 1 tablet: PhosphoStop	-20C
	10X cOmplete	1ml of MilliQ; 1 tablet: cOmpleteMini	-20C

A	B
1% TritonX-100 (1mL)	800uL TBS, 100uL 10X cOmplete, 100uL 10X PhosphoStop, 10uL TritonX-100 (1mL) 800uL TBS, 100uL 10X cOmplete,



A	B
	100uL 10X PhosphoStop, 10ul TritonX-100 0.0008g NaF

Working solutions (w/ PPIs)

Make and use the day of the extraction, keep on ice

Protocol

- 4 Collect 20-40 fly heads via Snap Freeze method into a Biomasher tube and transfer tubes to CTRND on dry ice.
- 5 Turn on Sorvall ultracentrifuge and set temperature to 4degC, place rotor inside and turn on vacuum to allow internal temperature to set.
- 6 Add 50uL (2.5uL/fly head) of 1% TritonX-100 with PPI to heads in Biomasher II tube and homogenize for 1.5 min with a hand-held automatic homogenizer and Biomasher pestles.
- 7 Briefly spin down in tabletop centrifuge to get the liquid to the bottom of the tube.
- 8 Sonicate at 35% with 5 one-second pulses for tissue to break down/dissolve. Skip this step for 'non-sonicated' extractions.
- 9 Clarify by centrifugation in a tabletop centrifuge (2,100g) for 15 seconds.
- 10 Move entire liquid sample into ThermoScientific 1.5ml microtube WX und MX (Cat #314352H01) before centrifuging
- 11 Centrifuge the supernatant at 100,000 g (29,900 rpm) for 30 min at 4°C using the chilled rotor.
- 12 Remove and save Supernatant into new labeled collection Eppendorf (soluble fraction).



- 13 Wash the pellet by adding 50uL of TritonX-100 with PPI and centrifuging at 100,000 g for 15 min at 4°C. Discard the supernatant.
- 14 Resuspend pellet in 50ul (2.5ul/fly head) in TritonX-100 with PPI by sonication with 3 one-second pulses or until it is fully resuspended. Label and save (insoluble fraction)
- 15 Perform BCA. If strapped on time, samples can wait to be quantified and stored at -80C.

Protocol references

<https://www.mdpi.com/1422-0067/25/7/3643>