APR 01, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.81wgbz151gpk/v1

Protocol Citation: ALFONSO.M.PENA 2024. SOP – 2-step protein fractionation (Triton) from fly heads. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbz151gpk/v1

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

Created: Apr 01, 2024

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

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ABSTRACT

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

PROTOCOL REFERENCES

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

Last Modified: Apr 01, 2024

PROTOCOL integer ID: 97609

Keywords: ASAPCRN

Funders Acknowledgement:
Martin Pena, Alfonso
Grant ID: NIH P40OD018537

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-1001% TritonX-100 (1mL) 800uL TBS, 100uL 10X cOmplete, 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.