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

Created: Apr 01, 2024

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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PROTOCOL integer ID: 97610

Keywords: ASAPCRN

Funders Acknowledgement:

Aligning Science Across Parkinson's (ASAP) initiative Grant ID: ASAP-020527

Reagents I	۷e	ed	ed	
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- 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)

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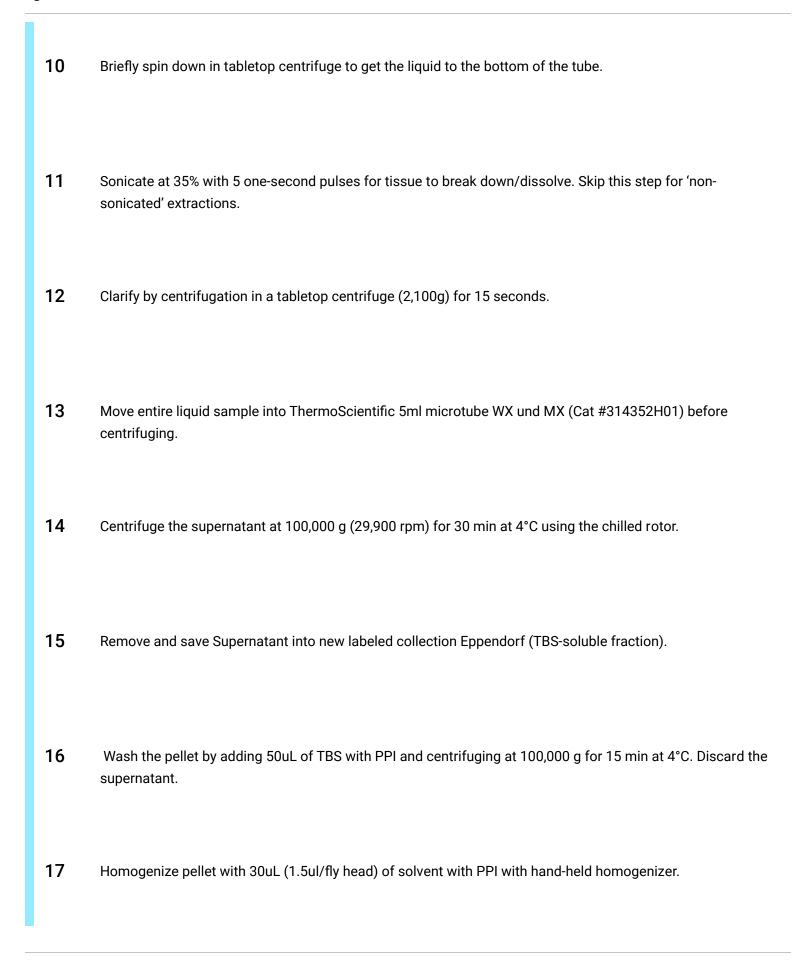
A	В	С
Stock Solutions	Formula	Storage
TBS	20 mM Tris Base, 150mM NaCl, pH 7.6	4C
20% SDS	10g SDS in 50ml of MilliQ	Room Temperature; tends to gel up, in which case you have to heat it up on a hot plate
RIPA Buffer	PierceTM, Thermo Scientific 89900	4C
10X PhosphoStop	1ml of MilliQ; 1 tablet: PhosphoStop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

A	В		
TBS	800uL TBS, 100uL 10X cOmplete, 100uL 10X PhosphoStop		
5% SDS	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	TRIBLIERS THEIR THE CHANNEL THEIR THE PROCEDUCION AND ALLE ALLE		
	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.
- 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.



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