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RNA and protein extraction from bulk dissections

In 1 collection

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ABSTRACT

mirVana PARIS RNA and Native Protein Purification Kit (#AM1556, Thermo Fisher Scientific)

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COLLECTIONS

In vivo reduction of age-dependent neuromelanin accumulation mitigates features of Parkinson's disease

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GUIDELINES

*We have less tissue than 0,5mg but 500 ul is a comfortable volume to work with since we have to homogenize the sample with a motorized rotor-stator homogenizer that needs a big volume to homogenize the whole sample. And, afterwards, we have to split the volume into RNA and Protein samples, so 500ul enables a 200 and 200ul separate homogenates (~100ul gets lost as foam) which is a correct volume.
** We suggest collecting only 200ul to avoid collecting interphase. But it is possible to collect 300ul from the aqueous phase if the interphase is compact.

SAFETY WARNINGS

Denaturing buffer contains β –mercapto so it has to be manipulated in an appropriate fume-hood.

First Time 10m

1 (1st time): Add 375ul of 2-mercaptoethanol to *2X Denaturing Solution*, Add 21ml of 100% Etanol to miRNA *Wash Solution 1*, Add 40ml of Etanol 100% to *Wash Solution 2/3*.

Sample disruption 30m

2 Preheat *Nuclease-free Water* (#129114, Qiagen-Werfen) at 95oC (for elution).

3 Preheat *2x Denaturing Solution* to 37 ^{o}C until it is completely in solution (to dissolve crystals formed when stored at 4 ^{o}C).

4 Keep bulk dissections in dry ice until adding to cell disruption buffer.

5 Add *Protease/phosphatase inhibitor cocktail 100x* (1/100) (#5872S, Werfen - Cell signaling) to *Cell disruption buffer*.

6 Add 500ul of *Cell disruption buffer* into 2mL eppendorfs. This volume can range from 100 to 625ul as stated in the protocol (~50mg tissue-->500 ul to each sample)* and keep the buffer on ice.

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7 Add the frozen bulk dissections to the 2mL eppendorfs with *Cell disruption buffer.*

8 Disrupt the samples using a *Omni Tissue Homogenizer (TH)*. Disrupt for no more than 5s and then put it back on ice, to keep the sample cold during homogenization. If you still see clumps of tissue homogenize 5s more. Try not to take the rotor out of the cell disruption buffer to avoid creating foam.

9 Split the sample for RNA and PROTEIN: 200ul RNA and 200ul Protein (approximately 100ul is lost as foam). Leave protein sample on ice for a minimum of 10 min or more, and proceed with the RNA sample.

RNA extraction 45m

10 Add *Denaturing Solution 2x*. Same volume as the sample split for RNA extraction (-->200ul). Denaturing buffer contains β –mercapto so it has to be manipulate din an appropriate fume-hood.

11 10. Incubate 5min on ice.

12 11. Add *Acid-Phenol: Chloroform*. Same volume as the actual sample (volume sample split for RNA extraction + volume *2x Denaturing Solution* (-->400 ul) (Take the bottom phase of the bottle of *Acid-Phenol:Chloroform*, not the aqueous phase on top).

13 12. Vortex 30-60s.

14 13. Centrifuge 8min at max. speed. If interphase is not compact centrifuge again.

15 14. Remove the upper aqueous phase (avoiding interphase) and transfer to a new tube**.

16 15. Add 1,25x volumes of Ethanol 100% (at room temperature).

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17 16. Pipette up and down 3 times and transfer to filter column (max. 700ul, if not perform successive transfers).

18 17. Centrifuge 30s at 10.000g. Discard flow-through.

19 18. Add 700ul *miRNA Wash Solution 1*. Centrifuge 15s at 10.000g. Discard flow-through.

20 19. Add 500 ul de *Wash Solution 2/3*. Centrifuge 15s at 10.000g. Discard flow-through(X2).

21 20. With the same tubes centrifuge 1min at 10.000g to eliminate residual fluid.

22 21. Transfer the column to a new *Collection tube* (provided). Add 30ul *Nuclease-free water* pre-warmed at 95oC to elute RNA.

23 22. Centrifuge 30s at 10.000g. Collect eluate.

24 23. Re-load the eluate in the column, centrifuge again 30s at 10.000g. Quickly place the samples on ice (RNA is now in water solution and is highly labile).

25 24. (Optional) Take 1,5ul from the extracted RNA sample for Bioanalyzer analysis.

26 25. (Optional) Take 1,5ul for RNA concentration quantification with NanoDrop ND-1000 Spectrophotometer.

27 26. As fast as possible store the samples at -80oC.

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Protein extraction 20m

28 27. Take the split sample for protein extraction and centrifuge at 4oC at 10.000g for 2min.

29 28. Collect the supernatant and transfer it to a new tube.

30 29. Sonicate 3x5min (30s on ice between intervals).

31 30. Store at -80oC.

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