RNA Extraction for RIN and DV 200 Analysis

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1 Works for me dx.doi.org/10.17504/protocols.io.86nhzde

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Human BioMolecular Atlas Program (HuBMAP) Method Development Community

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ABSTRACT

Scope: Extract RNA for RIN and DV 200 Assessment.

Expected Outcome: RIN and DV 200 measurements for tissue quality assessment.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Adapted from: Masato Hoshi, MD, PhD, Jain Lab, Division of Nephrology, Washington University School of Medicine

MATERIALS TEXT

Materials
1. Forceps
2. 1.5mL microcentrifuge tubes
3. 23G and 27G needle syringes
4. TRIzolLS
5. DEPC treated water
6. RNase free water
7. Phase Lock Gel, 5PRIME #2302830
8. Chloroform
9. Centrifuge
10. Isopropanol
11. Ethanol
12. Qiagen RNeasy Plus Micro Kit
13. 2-ME
14. RPE Buffer
15. Bioanalyzer

Solutions:
1. Glycogen Solution
5 mg/mL

Protocols:
Qiagen RNeasy Plus Micro Kit

Homogenize Tissue

1 Place tissue section in 750 µL of TRIzolLS.

2 Homogenize the sample:
Use a homogenizer or pass sample through a 23G then a 27G needle syringe.

3 Freeze samples on dry ice and re-thaw once. Mix well by vortexing.

4 Add RNase free water to a final volume of 960 µL.

5 Add 40 µL of glycogen solution (5mg/mL, making a final conc of 200 µg/mL of glycogen) to each tube and mix well.

Loading

6 Incubate for 5 minutes at 20^{O}C (room temperature).

7 Add 250 µL of chloroform and shake for 15 seconds.

8 Centrifuge at 12,000 x g for 10 minutes at 4^{O}C.

9 Move supernatant to a new 1.5 mL tube.

10 Add 600 µL isopropanol.

11 Vortex and incubate the tube at -20^{O}C for 20 minutes.

12 Centrifuge at 20,000 x g for 20 minutes at 4^{O}C.

13 Discard supernatant and add 600 µL 80% Ethanol.

14 Vortex and centrifuge at 20,000 x g for 5 minutes at 4^{O}C

15 Discard supernatant and air dry for a few minutes.

16 Add 300 µLof RLT plus buffer (from Qiagen RNeasy Plus Micro Kit) containing 2-ME (10 µL of 2-ME for 1 mL of RLT Plus Buffer, 1% 2-ME) and mix.

17 Add 450 µL 100% ethanol (1.5 volumes) and mix well.

18 Transfer 750 µLof the sample to RNeasy MinElute spin column and centrifuge for 1 min at 12,000 x g at 24^{O}C.

Collection

19 Centrifuge Phase Lock Gel to move gel to the bottom of column (12,000 x g for 5 minutes).
Then transfer the TRIzol mixture mixture to the column.

20 Add 500 µL of RPE buffer to the column and centrifuge at 12,000 x g for 1 min at 24^{O}C.

21 Discard flow through and add 500 µL RPE buffer to the column. Centrifuge at 12,000 x g for 2 min at 24^{O}C.

22 Transfer column to a new collection tube. Discard the old collection tube with the flow through.

23 Transfer the collected flow through back through the same column and centrifuge for 1 min at 12,000 x g at 24^{O}C. (This is a double application of sample). This time discard the flow.

24 Open the lid of the column and centrifuge at 12,000 x g for 5 min at 24^{O}C to dry up the column membrane completely.

25 Place the column in a new 1.5 mL tube and add 10 uL of RNase free water on the center of the column membrane. Incubate for 2 min at 20^{O}C.

26 Centrifuge at 12,000 x g for 3 min at 24^{O}C.

27 Add 10 uL of RNase free water again on the center of the column membrane and incubate for 2 min at 20^{O}C.

28 Centrifuge at 12,000 x g for 3 min at 24^{O}C (this is a double elution).

29 Total volume should be ~20 uL.

30 Provide VANTAGE Core with 10 uL of solution for RIN and DV 200 analysis.