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Department of Genomic Medicine

Cancer Immune Monitoring and Analysis Center

Division of Cancer Medicine

The University of Texas MD Anderson Cancer Center

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
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Director:

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SOP Title: *RNA Extraction from Frozen Tissue via QIAamp RNeasy Mini kit.*

I. Purpose

The purpose of this document is to provide instructions to extract RNA from Frozen tissue using QIAamp RNeasy Mini Kit.

II. Materials

1. Sterile RNase-free pipette tips.
2. Sterile Eppendorf tubes.
3. Ethanol (70%).
4. QIAamp DNA Blood Mini Kit: Buffer RLT, Buffer RPE, Buffer RW1, Buffer RPE, Buffer RDD, DNase I solution.
5. QIAamp Mini Spin Columns, QIAshredder spin column, sterile 20-gauge needle and syringe.

III. Preparation of reagents

1. Buffer RLT: Add 10uL of β-mercaptoethanol to 1mL of Buffer RLT. Make sure to prepare the reagent under a chemical fume hood with appropriate PPE.
2. Buffer RPE: Add 44mL of ethanol (96-100%) to 11mL of Buffer RPE (1 time).
3. DNase I solution: Dissolve the DNase stock I solution in 550uL of RNase free water, aliquot 10uL into small eppendorf tubes for long term storage at -20 °C for up to 9 months and store.
4. DNase I incubation mix: Add 70uL of RDD buffer to 10uL of the DNase I solution and place on ice.

IV. Notes

1. The following procedure is employed to extract RNA from frozen tissue.
2. Make sure to clean the bench surface, pipettes and equipment with RNaseZap to eliminate RNase contamination.

3. The amount of tissue cannot be over 25mg or it will overload the spin column which can greatly reduce the RNA yield.

V. Procedure

1. Collect enough dry ice to fill the ice bucket ¾ full.
2. Clean tweezers and spatulas thoroughly with 70% ethanol and allow them to dry.
3. Label the required number of weigh boats and place in container filled with dry ice.
4. Once the weight boat is frozen, place it on a precision balance and zero out the scale.
5. Place the frozen tissue in the frozen weigh boat and weigh again, determining the amount of tissue.
6. If there is more than 25 mg of tissue, cut the tissue and use a smaller portion. Tissue larger than 25mg will hinder the extraction.
7. Add the frozen tissue to a 2-mL screw-top tube and add 350uL Buffer RLT. Homogenize the tissue until it is broken into small pieces.
8. Transfer lysate to a microcentrifuge tube and centrifuge at full speed for 3 min. Carefully remove the supernatant by pipetting and transfer to another new microcentrifuge tube. Use only this supernatant/lysate in the subsequent step.
9. Add 350uL (1volume) of 70% ethanol to the homogenized lysate, mix well by pipetting (Do not vortex).
10. Transfer up to 700uL of the sample to the RNeasy spin column placed in a 2mL collection tube. Centrifuge for 30 seconds at 9550 rpm. Discard the flow through.
11. Transfer the RNeasy spin column to a new 2mL collection tube and proceed to the DNase I digestion for eliminating the genomic DNA contamination:
12. DNase Digestion
    i. Add 350uL Buffer RW1 to the RNeasy spin column. Centrifuge for 30 seconds at 9550 rpm to wash the spin column membrane. Discard the flow through.
    ii. Add 10uL DNase I stock solution to 70uL Buffer RDD. Mix by slowly pipetting or inverting the tube. Keep the mixture on ice.
    iii. Add 80uL of the DNase I incubation mix directly to the RNeasy spin column membrane, and incubate at room temperature for 15min.
    iv. Add 350uL Buffer RW1 to the RNeasy spin column. Centrifuge for 30 seconds at 9550 rpm. Discard the flow through and transfer the RNeasy spin column to a new 2mL collection tube.
13. Add 500uL Buffer RPE to the RNeasy spin column and incubate at room temperature for 5min. Centrifuge for 30 seconds at 9550 rpm. Discard the flow through and transfer the RNeasy spin column to a new 2mL collection tube.

14. Add 500uL Buffer RPE to the RNeasy spin column. Centrifuge for 2 minutes at 9550 rpm. Discard the flow through and transfer the RNeasy spin column to a new 2mL collection tube.
15. Centrifuge the RNeasy spin column for 1 minute at full speed to eliminate possible carryover of the Buffer RPE.
16. Place the RNeasy spin column in a new 1.5mL collection tube (supplied). Add 50uL of RNase-free water directly to the spin column membrane. Centrifuge for 1 minute at 12,000 rpm to elute the RNA and vortex.
17. Place the eluted RNA immediately on ice and store appropriately.

18. **Definitions**

N/A

19. **Related Documents:**

Environmental Protection Website:
http://inside.mdanderson.org/departments/facilities/emergency-safety/environmental-protection.html

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