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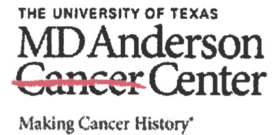

Department of Genomic Medicine

Cancer Immune Monitoring and Analysis Center

Division of Cancer Medicine

The University of Texas MD Anderson Cancer Center

Director:

Andy Futreal, BS, PhD,Pro fessor and Chair

SOP Title: ***Extraction of RNA from FFPE Tissue Sections.***

I. Purpose

The purpose of this document is to provide instructions for the extraction of RNA from FFPE tissue sections using Qiagen RNeasy FFPE Kit.

II. Materials

1. Xylene.
2. Ethanol (96%-100%).
3. Water bath @ 56˚C & heating block @ 80˚C.
4. RNeasy FFPE Kit: Buffer RBC, Buffer PK, Proteinase K, RNase-Free DNase I (lyophilized), RNase-Free Water, DNase Booster Buffer, Buffer RPE.
5. RNase A (100ng/mL).
6. RNeasy MinElute Spin Columns (pink) & 2mL collection tubes.

III. Notes

1. The following procedure is employed to extract RNA from FFPE tissue sections using Qiagen's RNeasy FFPE Kit.
2. The user must supply 100% ethanol and Xylene.
3. Prepare DNase I stock solution by dissolving the lyophilized DNase I in 550ul of RNase-free water. To avoid loss of DNase I, do not open the vial. Inject RNase-free water into the vial using a needle and syringe. Mix gently by inverting the vial. Do not vortex.
4. To prepare Buffer RPE, add 4 volumes (44ml) ethanol (96-100%) to the bottle containing 11ml Buffer RPE concentrate. Before starting the procedure, mix reconstituted Buffer RPE by shaking.
5. Chemical waste should never be poured down the sink.

IV. Procedure

1. Scrape the FFPE tissue from the slides using a clean razor and pair of tweezers. Move the tissue into a labeled, 1.5-mL microcentrifuge tube.
2. In the fume hood, add 1mL xylene. Invert the tubes to mix – do not vortex.
3. Incubate for 30 minutes.
4. Centrifuge at full speed for 3 minutes at room temperature.
5. Remove supernatant, careful not to disturb the pellet.
6. Repeat steps 1-3 until the FFPE sample loses its structural integrity, which typically takes about 1-2 intervals.
7. Add 1mL ethanol and incubate for 30 minutes.
8. Centrifuge at full speed for 3 minutes at room temperature.
9. Remove supernatant i.e., carefully remove residual ethanol using a fine pipet tip.
10. Open the tube and incubate sample at room temperature for 10 minutes or until all residual ethanol has evaporated. Larger pellets will take longer to dry – close the tube caps as the pellets dry to prevent additional drying.
11. Re-suspend the pellet in 150µL Buffer PKD and mix by vortexing. Centrifuge for 1 minute at 10,000 rpm.
12. Add 10ul proteinase K to the lower, clear phase. Mix gently by pipetting up and down.
13. Incubate at 56˚C for approximately 15 minutes or until the sample has been completely lysed.
14. Incubate at 80˚C for 15 minutes. If a heating block without a shaking function is used, briefly mix by vortexing or "flicking" the tube every 3-5 minutes. Ensure that the heating block has reached 80˚C before starting the incubation.
15. Transfer the lower, uncolored phase into a new 2ml microcentrifuge tube.
16. Incubate on ice for 3 minutes. Then, centrifuge for 15 minutes at 13,500 rpm.
17. Transfer the supernatant to a new microcentrifuge tube taking care not to disturb the pellet.
18. Add DNase Booster Buffer equivalent to a tenth of the total sample volume (approximately 16ul) and 10ul DNase I stock solution. Mix by inverting the tube. Centrifuge briefly to collect residual liquid from the sides of the tube.
19. Incubate at room temperature for 15 minutes.
20. Add 320ul Buffer RBC to adjust binding conditions and mix the lysate thoroughly.
21. Add 720ul ethanol (100%) to the sample, and mix well by pipetting. Do not centrifuge. Proceed immediately to step 20.
22. Transfer 700ul of the sample, including any precipitate that may have formed, to an RNeasy MinElute spin column placed in a 2 ml collection tube. Close the lid gently and centrifuge for 30s at 10,000rpm. Discard the flow-through.
23. Add 500ul Buffer RPE to the RNeasy MinElute column. Close the lid gently and centrifuge for 2 minutes at 10,000rpm to wash the spin column membrane. Discard the collection tube with the flow-through.

24. Place the RNeasy MinElute spin column in a new 2ml collection tube. Open the lid of the spin column and centrifuge at full speed for 5 minutes. Discard the collection tube with the flow-through.
25. Place the RNeasy MinElute spin column in a new 1.5 ml collection tube. Add 30ul of RNase –free water directly to the spin column membrane and centrifuge for 1 minute at 10,000 rpm.
26. Centrifuge for 1 minute at 10,000rpm to elute the RNA.
27. RNA should be stored at -80°C.

V. Definitions

N/A

VI. Related Documents

Genomics Laboratory

Curtis Gumbs, Scientific Manager, Genomic Medicine

[Signatures] and [dates] 7/18/18