UCSF Brain Tumor Center

UCSF Brain Tumor Center SPORE Biorepository and Biomarkers Laboratory

Standard Operating Procedure: RNA extraction from FFPE tissue*

1. Select regions of interest from H&E stained slide.
2. Punch block using 1.5 mm diameter biopsy punch.
3. For blocks with > 0.5 mm tissue thickness, 1 core is enough (assuming good viable tumor cellularity without much artifact/necrosis). For blocks with <0.5 mm thickness, take 2 punches.
4. Place tissue in 1.5 mL microcentrifuge tube and grind tissue using micropestle. This step is very important and often the number of samples attempted has to be limited to </=12.
5. Add 320 µl xylene, vortex vigorously for about 10 sec, and centrifuge briefly at max speed to bring sample to the bottom of the tube. Sometimes, even after vortexing vigorously the sample remains stuck to the wall of the tube. To avoid this problem, add xylene and use the pipette tip to dislodge the paraffin from the wall.
6. Incubate at 56°C for 3 min. on a shaker (1000RPM) with manual shaking every minute.
7. Allow sample to cool at room temperature for 15 min.
8. Add 240µL Buffer PKD
9. Centrifuge for 1 min at 11,000 x g (10,000 rpm)
10. Add 10µL proteinase K to the lower, colorless phase. Mix gently by pipetting up and down.
11. Incubate at 56° C for 15 min. If tissue debris is visible after 15 min at 56°C then incubation can be prolonged up to 3 hours. Small amounts of insoluble material are permitted.
12. Incubate at 80°C for 15 min. It is essential to preheat the heating block to 80°C. Incubation of RNA at 80°C is critical for reversal of crosslinks and optimal RNA performance in downstream applications. The incubation in buffer PKD at 80°C for longer than 15 min can result in more fragmented RNA. For some applications it may be a balance between fragmented RNA and improved reversal of formalin modifications.

*Adapted by Gerald Reis from User-Developed Protocol for RNeasy Kit (RY42 Purification)