Promega

Product Application

Total RNA and miRNA purification from FFPE samples using the ReliaPrep™ FFPE Total RNA Miniprep System

Purify total RNA, including miRNA, from FFPE tissues manually.

Kit: ReliaPrep™ FFPE Total RNA Miniprep System (Cat. #Z1001)

Analyses: NanoDrop® quantitation
mRNA amplification (B2M)
GoTaq® 1-step RT-qPCR System for Dye-based Detection
miRNA amplification (miR-16, miR-29a)
TaqMan™ MicroRNA Reverse Transcription Kit
GoTaq® Probe qPCR Master Mix
gDNA contamination (B2M), GoTaq® qPCR Master Mix

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

Further information can be found in Technical Manual #TM353, available at: www.promega.com/protocols

Sample Type(s): Formalin-fixed paraffin-embedded (FFPE) tissue samples

Input: 10µm FFPE tissue sections (in microcentrifuge tubes)

Materials Required:

- 100% Isopropanol
- 95-100% Ethanol
- 80°C heat block
- 56°C heat block
- Benchtop centrifuge

Protocol:

Follow the protocol in the ReliaPrep™ FFPE Total RNA Miniprep System Technical Manual (#TM353) through step 1 of section 6.D, including addition of BL Buffer to the sample.

1. Add 570µl of 100% Isopropanol. Vortex briefly to mix.
2. Centrifuge at 10,000 *x g* for 15 seconds at room temperature.
3. For each sample processed, place a Binding Column into one of the provided Collection Tubes.
4. Transfer 700µl of the lower, blue phase to the Binding Column.
5. Cap the column and centrifuge the assembly at 10,000 *x g* for 30 seconds at room temperature.
6. Discard the flow-through and add the remaining aqueous phase to the column.
7. Cap the column and centrifuge the assembly at 10,000 *x g* for 30 seconds at room temperature.

Proceed with section 7 of the Technical Manual protocol (Column Washing and Elution).

Results:

Four 10µm sections of normal mouse FFPE liver were purified as described above using the ReliaPrep™ FFPE Total RNA Miniprep System. Eluates were adjusted to 50µL with nuclease-free water and purified nucleic acid was quantified by absorbance, mRNA amplification (B2M), miRNA amplification (miR-16 and miR-29a), and amplification of contaminating gDNA. All purification reactions resulted in substantial quantities of mRNA and miRNA targets with no detectable gDNA contamination.

Table 1. Analysis of total RNA eluates purified from 10µm sections of mouse FFPE liver. After purification, samples were analyzed by absorbance on a NanoDrop® One. Samples were diluted 1/20 and 2µL was amplified with GoTaq® 1-step RT-qPCR System with B2M RNA-specific primers; or in qPCR with GoTaq® qPCR Master Mix with B2M DNA-specific primers. Samples were also diluted 1/100 and 5µL was reverse transcribed with TaqMan™ MicroRNA Reverse Transcription Kit with the human/murine hsa-miR-16 and hsa-miR29a assays, then amplified with GoTaq® Probe qPCR Master Mix. Mean results with standard deviation are given (n=4).

Analysis	Result
Quantification - absorbance	196 ± 15 ng/µL
Yield – absorbance	9.80 ± 0.75µg
Purity – A260/A280	1.88 ± 0.01
Purity – A260/A230	1.13 ± 0.08
mRNA Amplification – B2M	Cq 21.21 ± 0.08 (0.1µL eluate)
miRNA Amplification – miR-16	Cq 24.56 ± 0.08 (0.05µL eluate)
miRNA Amplification – miR-29a	Cq 25.16 ± 0.08 (0.05µL eluate)
gDNA Amplification – B2M DNA	No amplification (64pg/µL assay sensitivity)