



MICRO RNA AND TOTAL RNA PURIFICATION FROM LIVER TISSUE

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CONTEXT

Micro RNAs are small naturally occurring non-coding ribonucleic acids with sizes between 18 and 40 nucleotides (nts) that have been demonstrated to play a significant role in the regulation of gene expression. As a result, interest in smaller RNA species, such as miRNA, has increased. Here we describe the purification of total RNA, including miRNA and other small RNA molecules from fresh frozen tissue samples using Beckman Coulter's Agencourt SPRI (Solid Phase Reverse Immobilization) magnetic bead based chemistry. Beckman's Agencourt RNAdvance tissue extraction kits (AA32646) and Biomek automation NX^P liquid handler (A31840) produced high quality miRNA and RNA using the Precellys homogenizer.

MATERIALS

- **Precellys24-Dual homogenizer**
- Lysing kit: CK28_7mL (KT03961-302.7)
- Samples: 50-100 mg of rat liver (4 replicates)
- Buffer: 1mL of RNAdvance Tissue lysis buffer (Beckman Coulter, A32646) and anti-foaming DX Reagent (Qiagen, 19088)

PROTOCOL

- **Precellys parameters:** 6500 rpm, one cycle of 20 sec, and 6000 rpm for a 2nd cycle for 20 sec
- Lysates were adjusted to 10 mg per 400 μ L lysis buffer and then digested with proteinase K at 37°C for 25 minutes, followed by RNA extraction using the RNAdvance Tissue miRNA protocol (www.beckman.com, AAG-230APP07.14-A and AAG-453APP08.14-A).
- RNA concentration was measured with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific).
- Let-7c miRNA gene expression was measured using the Taqman microRNA assay (Life Technologies, 4427975).

RESULTS

Liver tissue replicates were used to evaluate miRNA extraction efficiency. The calculated average yield prepared from the RNAdvance Tissue Kit was between 25-30 μ g per 5 mg liver tissue (Table 1). The average cycle threshold (Ct) value for let-7c miRNA expression in RNA samples was 25 cycles (Figure 1).

Method	Yield per 5 mg liver tissue (μ g)	Average yield per 5 mg liver tissue (μ g)
RNAdvance Tissue	25-30	27.80 \pm 2.2

Table 1. The average RNA yield prepared from the Precellys homogenizer

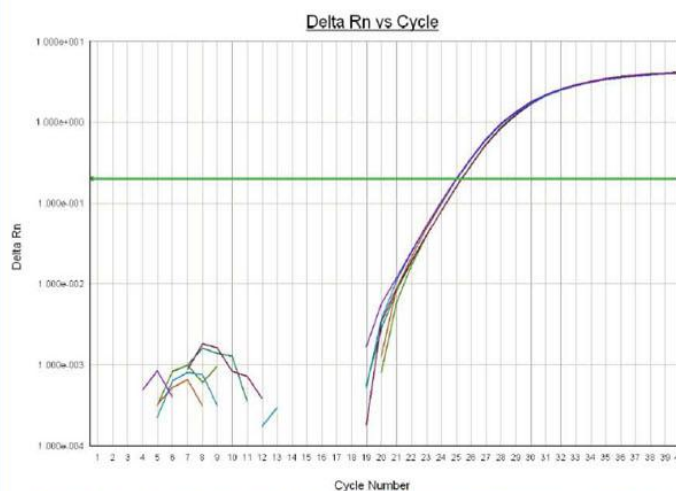


Figure 1. Overlay of let-7c gene expression from the RNAdvance Tissue Kit

CUSTOMER



Beckman reference #: AAG-241APP07.14-A
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CONCLUSION

The data shows that the RNAdvance Tissue Kit provides high quality RNA and miRNA when coupled with the Precellys homogenizers. At the same time, multiple samples can be run simultaneously in either 2mL or 7mL tubes, which saves a significant amount of time during the sample preparation process. Reproducible RNA yields are also observed between replicates, and cross-contamination between samples is eliminated with the use of Precellys pre-filled lysing tubes.

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