Tips from the bench

Stopping point: lysate storage

Introduction

A convenient stopping point is an important part of any RNA isolation workflow, but it is critical to ensure that stopping the workflow doesn't negatively impact RNA integrity and recovery efficiency. While developing the Applied Biosystems™ MagMAX™ *mir*Vana™ Total RNA Isolation Kit (Cat. No. A27828), we included an ideal stopping point after the lysis step that gives users greater flexibility. For each protocol developed for the kit, we tested storage of sample lysates at –20°C for up to 4 days and observed no change in RNA integrity, recovery efficiency, or gene expression compared to samples that were fully processed immediately after lysis.

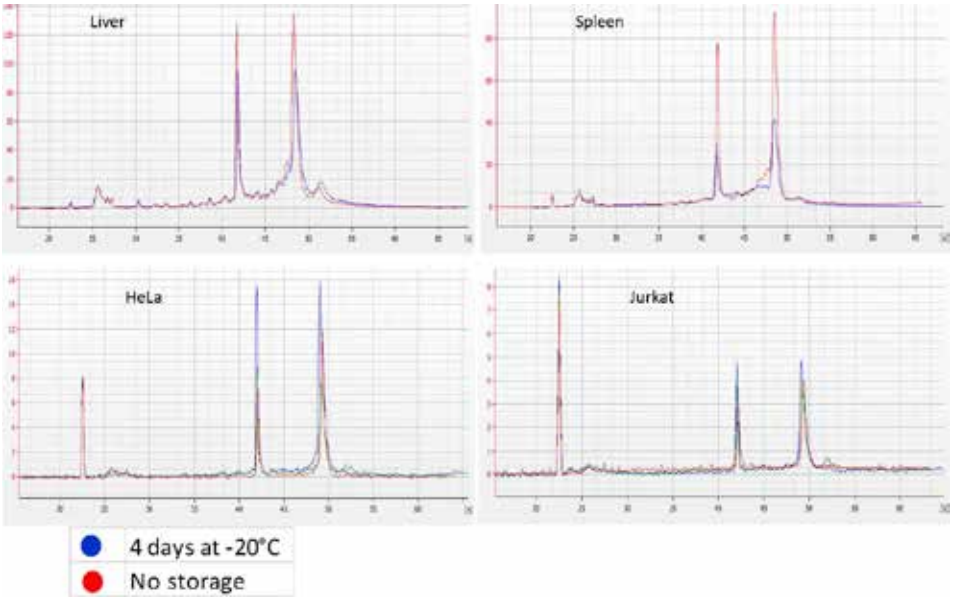

Figure 1. Analysis of RNA quality. Total RNA, including small RNA, was isolated with the MagMAX *mir*Vana Total RNA Isolation Kit from fresh sample lysates or after storage at –20°C for 4 days. Equal volumes of each were then run on the Agilent™ 2100 Bioanalyzer™ instrument. The electropherograms above are nearly identical between the two methods, demonstrating minimal loss of RNA integrity due to storage.

RNA integrity analysis

RNA was isolated from mouse tissues, HeLa cells, and human biofluids (plasma, serum, and urine) using the MagMAX *mir*Vana Total RNA Isolation Kit and protocols specific for each sample type. For each protocol, once samples were lysed, half of the lysate was stored at –20°C while the other half was immediately processed. After 4 days (to mimic storage of the samples over the weekend), the frozen lysates were thawed and RNA isolated using the same protocols as for the nonfrozen lysates. As shown in Figure 1, minimal differences in RNA integrity were seen for the stored lysate samples.

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qRT-PCR analysis

Equal volumes of RNA input from each sample (diluted 1:10 for miRNA analysis of tissues and cells) were used for cDNA synthesis. cDNA was generated using the Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit for mRNA analysis and the Applied Biosystems™ TaqMan™ MicroRNA Reverse Transcription Kit and specific primers for miRNA analysis. qPCR reactions were then performed with 2 µL of cDNA using Applied Biosystems™ TaqMan™ Assays and Applied Biosystems™ TaqMan™ Universal Master Mix II, no UNG. Threshold cycle (C_t) values were averaged and then graphed by target (Figure 2). Panel A shows results from biofluid samples, while Panel B shows results from tissues and cells. Results demonstrate minimal, if any, change in gene expression due to storage.

A B

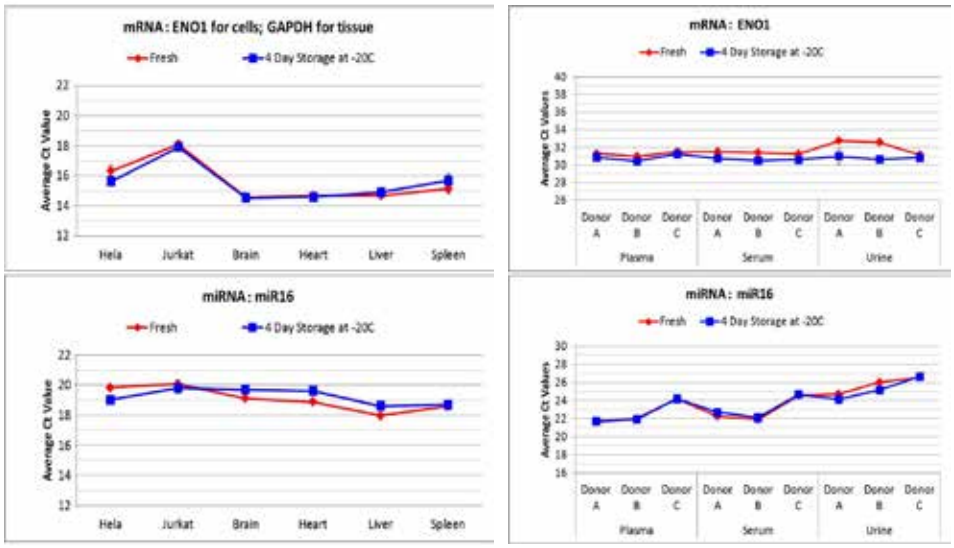

Figure 2. Analysis of mRNA and miRNA by qRT-PCR. Total RNA, including small RNA, was isolated with the MagMAX *mir*Vana Total RNA Isolation Kit from plasma, serum, brain, heart, liver, spleen, urine, and two cell lines (HeLa and Jurkat) immediately or from lysates stored for 4 days at –20°C. RNA was reverse-transcribed and then analyzed by qPCR. Results demonstrate minimal or no difference in gene expression due to storage.

Conclusions

As demonstrated in this study, storing samples as lysates at –20°C has no adverse effects on RNA integrity and provides a convenient stopping point for users. Not only can lysates be stored over the weekend (as represented in this study by four days of storage), but separate experiments have demonstrated that samples can be stored for at least a month without impacting RNA integrity (data not shown). The MagMAX *mir*Vana Total RNA Isolation Kit provides a simple and flexible protocol for users, as well as chemistry that stabilizes and preserves RNA over time.

Find out more at **thermofisher.com/magmaxmirvana**

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