Application Note

Isolation of RNA from Animal Tissues using the PowerLyzer™ UltraClean® Tissue & Cells RNA Isolation Kit

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Introduction

The first step in the isolation of RNA from animal tissues is a strong homogenization sufficient to break down fibrous cellular matrices to allow for the fast neutralization of RNases during the extraction process. Methods for homogenization include the use of liquid nitrogen, hand held rotor-stators, and the use of bead-based homogenizers that can process multiple samples simultaneously. With bead-based homogenizers, the method must be fast enough to break down samples without overheating or degrading the RNA. The use of the high-velocity PowerLyzer™ 24 homogenizer with 2.8 mm ceramic beads allows for fast and efficient RNA extraction from a variety of tissues with high integrity and yields. The PowerLyzer™ UltraClean® Tissue & Cells RNA Isolation Kit provides a complete method for the purification of total RNA from any tissue type using the optimal 2.8 mm ceramic beads, pre-loaded into bead tubes, and ready to use on any bead-based homogenizer. Protocols for using the kit with the PowerLyzer™ 24 homogenizer provide a fast and easy optimization-free method for obtaining high quality total RNA without the use of phenol based reagents.

Methods

The PowerLyzer™ UltraClean® Tissue & Cells RNA Isolation Kit (cat. no. 15055-50) was used as directed in combination with the On-Spin Column DNase I Kit (cat no. 15100-50). The PowerLyzer™ 24 homogenizer (cat. no. 13155) was run at the speeds indicated in each legend (Figure 1) for two pulses of 45 seconds with a 30 second rest between pulses. The Precellys® was run at the optimized protocol of 3 cycles at 6500 RPM for 45 seconds with a 20 second rest between pulses. Two microliters of each eluate was electrophoresed on 1% agarose gels **(Figures 1 and 2)**.

Results

The PowerLyzer™ 24 Homogenizer Extracts RNA Faster than the Precellys®

The isolation of RNA was compared between the PowerLyzer™ 24 Homogenizer and the Precellys® 24 homogenizer for speed in macerating mouse liver, an RNA-rich tissue. The optimized protocol for the Precellys® was used (6500 RPM, 3 cycles at 45 seconds) and compared to two different speed settings on the PowerLyzer™ 24 homogenizer (3500 RPM and 4200 RPM) for 2 cycles at 45 seconds **(Figure 1)**. Due to the horizontal positioning and more efficient acceleration of beads within the bead tubes, complete RNA extraction resulted in less time uisng the PowerLyzer™ 24 homogenizer. The ability to reduce the homogenization to just two cycles prevents RNA degradation caused by excess heat generation from the high-velocity movement of beads within the tubes.

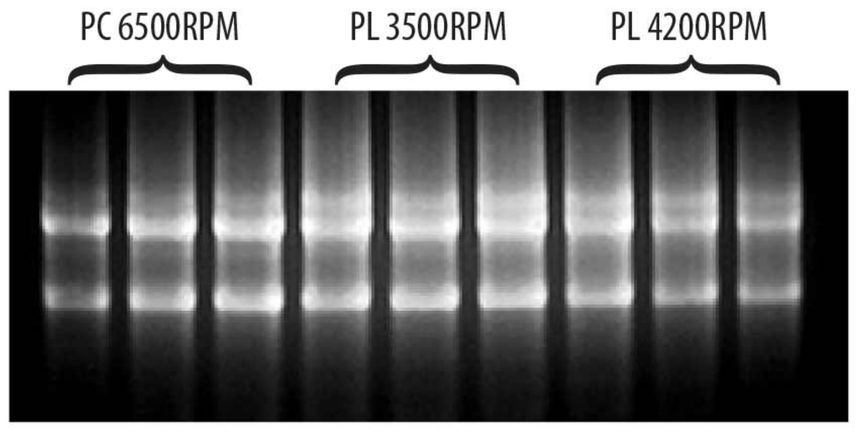

Figure 1. Isolation of total RNA from 10 mg of mouse liver tissue using the PowerLyzer™ UltraClean® Tissue & Cells RNA Isolation Kit. Using the PowerLyzer™ 24 homogenizer, faster acceleration of beads results in more efficient lysis with less cycles. Optimal RNA yields and recovery were obtained at the lower speed of 3500 RPM for 2 cycles of 45 seconds.

The PowerLyzer™ 24 Homogenizer Extracts RNA from a Variety of Animal Tissues

Since 3500 RPM was determined to be the optimal setting for RNA extraction from mouse liver, this speed was used to process a range of different mouse tissues including difficult to extract samples such as muscle and heart. RNA was isolated with excellent quality and expected yields from all tissues tested. The PowerLyzer™ 24 homogenizer was successful in extracting RNA from both soft and fibrous tissues (**Figure 2**).

The PowerLyzer™ 24 Homogenizer efficiently generates high quality RNA from a variety of mouse tissues

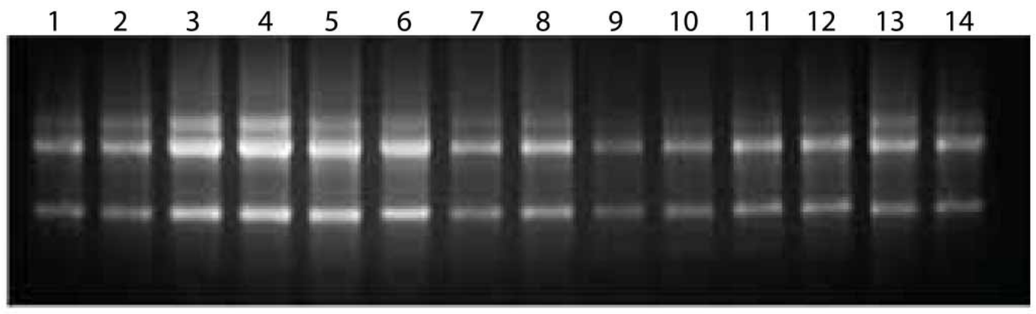

Figure 2. Mouse tissues (8 mg liver (1,2), and spleen (3,4), 10 mg kidney (5,6), and 12 mg lung (7,8), heart (9,10), brain (11,12), and muscle (13,14)) were homogenized in 2.8 mm ceramic bead tubes using the PowerLyzer™ 24 homogenizer for two 45-second pulses with a 30-second rest between pulses at 3500 rpm. RNA was extracted using the PowerLyzer™ UltraClean® Tissue & Cells RNA Isolation Kit with the On-Spin Column DNase I Kit as directed. Two microliters of each eluate were electrophoresed on a 1% agarose gel. Results indicate high quality and yields of RNA obtained for all samples tested.

Summary

There are many choices for the isolation of RNA from tissue samples that are effective at macerating tissues. When processing multiple samples, the most effective and fastest method is with the PowerLyzer™ 24 Bench Top Bead-Based Homogenizer. By reducing the cycle numbers required for complete homogenization, samples are subjected to less heat generation and exposed faster to the RNase inhibiting chemistry that protects RNA from degradation. By combining the optimized PowerLyzer™ UltraClean® Tissue & Cells RNA Isolation Kit with the PowerLyzer™ 24 homogenizer, high quality total RNA is obtained from the toughest samples in less time and with less degradation compared to traditional homogenization methods.

Catalog No.	Description	Quantity
13155	PowerLyzer™ 24 Bench Top Bead-Based Homogenizer, (110/220V)	1 unit
13156	PowerLyzer™ Tube Holder	1 unit
13157	PowerLyzer™ Tube Holder Stand	1 unit
15055-50	PowerLyzer™ UltraClean® Tissue & Cells RNA Isolation Kit	50 preps
13114-50-CBT	PowerLyzer™ Ceramic Bead Tubes, 2.8 mm	50 tubes
13114-325	Ceramic Beads, 2.8 mm, Bulk (500 preps, 325g)	325 g