APPLICATION NOTE

High-throughput RNA extraction from *Mus musculus* tissues using a tissue RNA kit and the Omni Bead Ruptor 12 bead mill homogenizer.

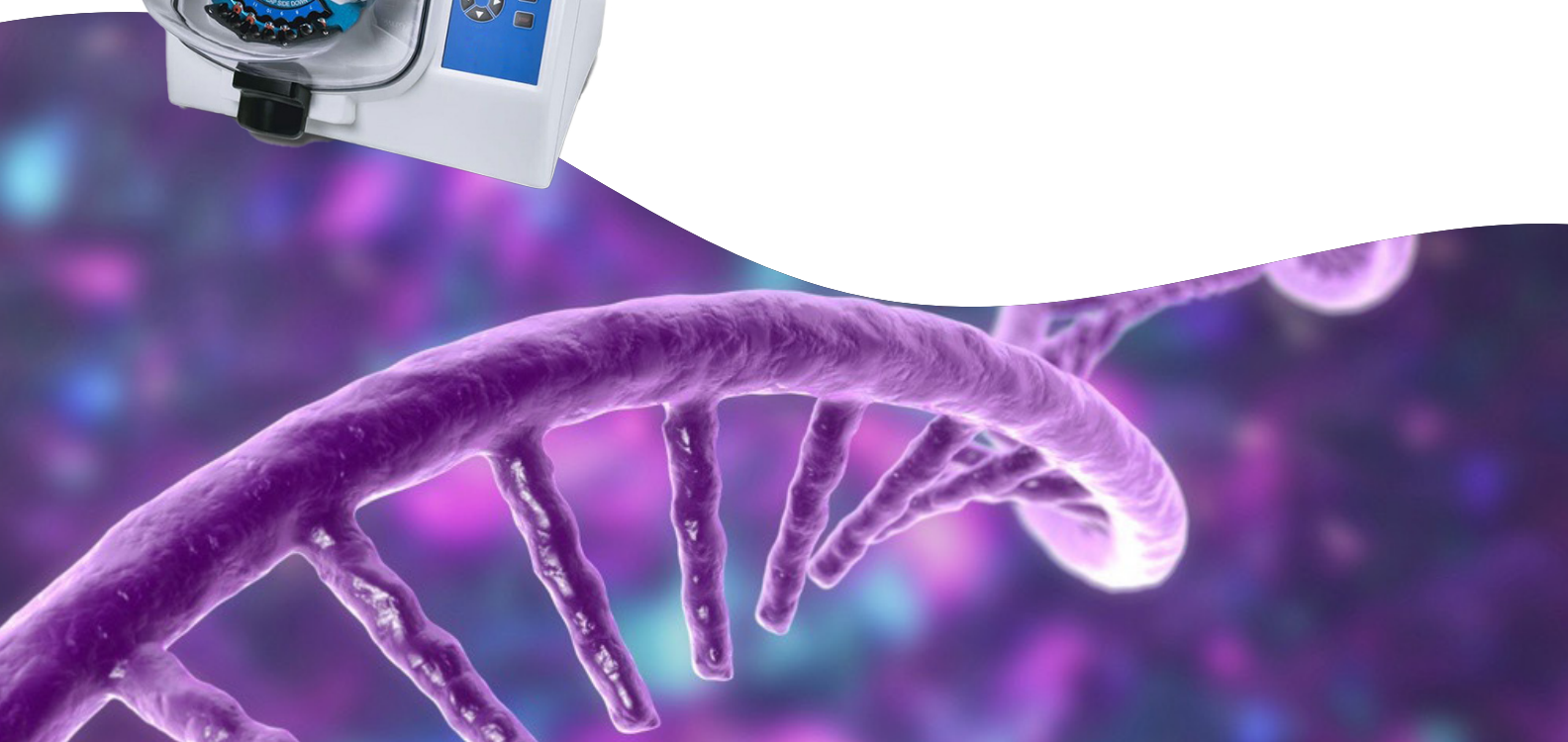
Omni Bead Ruptor 12 bead mill homogenizer

Introduction

The extraction and isolation of RNA is an integral part of downstream analyses such as RT-PCR, RT-qPCR, Northern blotting, and cDNA library construction. The importance of using pure, intact RNA for these processes is well documented and a critical part of downstream analysis success. It is well known that RNA is sensitive to degradation due to mechanical shear, temperature, storage conditions and freeze-thawing. Furthermore, RNA is highly susceptible to RNAse degradation following release of nucleases during the tissue disaggregation process. Thus, proper sample handling during the homogenization process is crucial when performing an RNA based assay.

Bead-based homogenization upstream of RNA extraction can help streamline the preparation of tissue when compared to manual methods. Herein, we demonstrate the Omni Bead Ruptor 12 bead mill homogenizer in a multi-tissue RNA workflow comparing bead based sample prep to manual cryomilling techniques. RNA integrity and yield were measured downstream and used to drive comparison between sample preparation methods.

For research use only. Not for use in diagnostic procedures.



Materials and methods

Equipment

- **Omni Bead Ruptor 12 bead mill homogenizer** (Cat # 19-050A)
- **Hard Tissue Homogenizing Mix 2.8 mm Ceramic (2 mL)** (Cat # 19-628)

RNA extraction

Fresh murine tissues (kidney, liver, heart, lung) were obtained. 25 mg of each tissue was placed in a pre-chilled 2 mL tube containing 2.8 mm ceramic beads and 500 µL of pre-chilled commercially available tissue RNA lysis buffer containing 2-mercaptoethanol. Samples were lysed on the Omni Bead Ruptor 12 bead mill homogenizer at 2.9 m/s for 20 seconds. As a control, 25 mg of each tissue was cryomilled in pre-chilled mortar & pestle under liquid nitrogen. The powdered tissue was then transferred to a pre-chilled 1.5 mL microcentrifuge tube including 500 µL of pre-chilled commercially available tissue RNA lysis buffer containing 2-mercaptoethanol. Following sample preparation, RNA was extracted and purified using a commercially available tissue RNA extraction kit following manufacturer's instructions. All steps were performed with extraction tubes on ice and using a centrifuge pre-chilled to 4 °C. An optional DNase treatment step was also performed on all samples following kit manufacturer guidelines for DNA removal. RNA was eluted in 100 µL DEPC water.

RNA quantification and integrity analysis

1 µL of purified RNA was analyzed, in triplicate, on an automated electrophoresis system as per manufacturer's instrument instructions and RNA kit protocol. Gel images, electrophoretograms, and RNA integrity numbers (RINs) were visualized and analyzed on the 2100 Bioanalyzer® Expert software (Agilent).

Results

Herein, we evaluated the Omni Bead Ruptor 12 to prepare tissue samples for extraction of high quality RNA as compared to a traditional cryomilling method. Table 1 shows the average RNA yield for each tissue type processed on the Omni Bead Rupter 12 with heart tissue exhibiting the lowest yields at 105.7 ng/µL and liver yielding RNA at concentrations of 733.3 ng/µL. The bead milling approach produced RNA yields in excess of 2X compared to the cryomilling method for liver, heart and lung tissues. RNA integrity numbers (RIN) were comparable for both the bead mill and cryomilling methods. Based on gel analysis, the RNA was of good quality with prominent 28S and 18S bands (Figures 1-2). There was no high molecular weight shearing visible in the electrophoretograms indicating the RNA was intact and ready for further downstream analyses (Figures 1-2).

Table 1: Average RNA yield for and RIN value for each tissue type processed on the Omni Bead Ruptor 12.

Tissue	Average yield	Average RIN
Kidney (mortar & pestle)	192.3 ng/µL	7.7
Liver (mortar & pestle)	167.3 ng/µL	7.5
Heart (mortar & pestle)	26.7 ng/µL	7.6
Lung (mortar & pestle)	44.7 ng/µL	7.2
Kidney (bead ruptor)	186 ng/µL	8.6
Liver (bead ruptor)	733.3 ng/µL	7.1
Heart (bead ruptor)	105.7 ng/µL	8.9
Lung (bead ruptor)	106 ng/µL	7.9

Conclusion

The Omni Bead Ruptor 12 was able to effectively lyse tissue samples, preparing tissue for downstream extraction of high quality RNA. RNA yields were higher with the bead milling approach and RNA integrity was maintained at acceptable levels when compared to cryomilling.

High-throughput RNA extraction from *Mus musculus* tissues using a tissue RNA kit and the Omni Bead Ruptor 12 bead mill homogenizer.

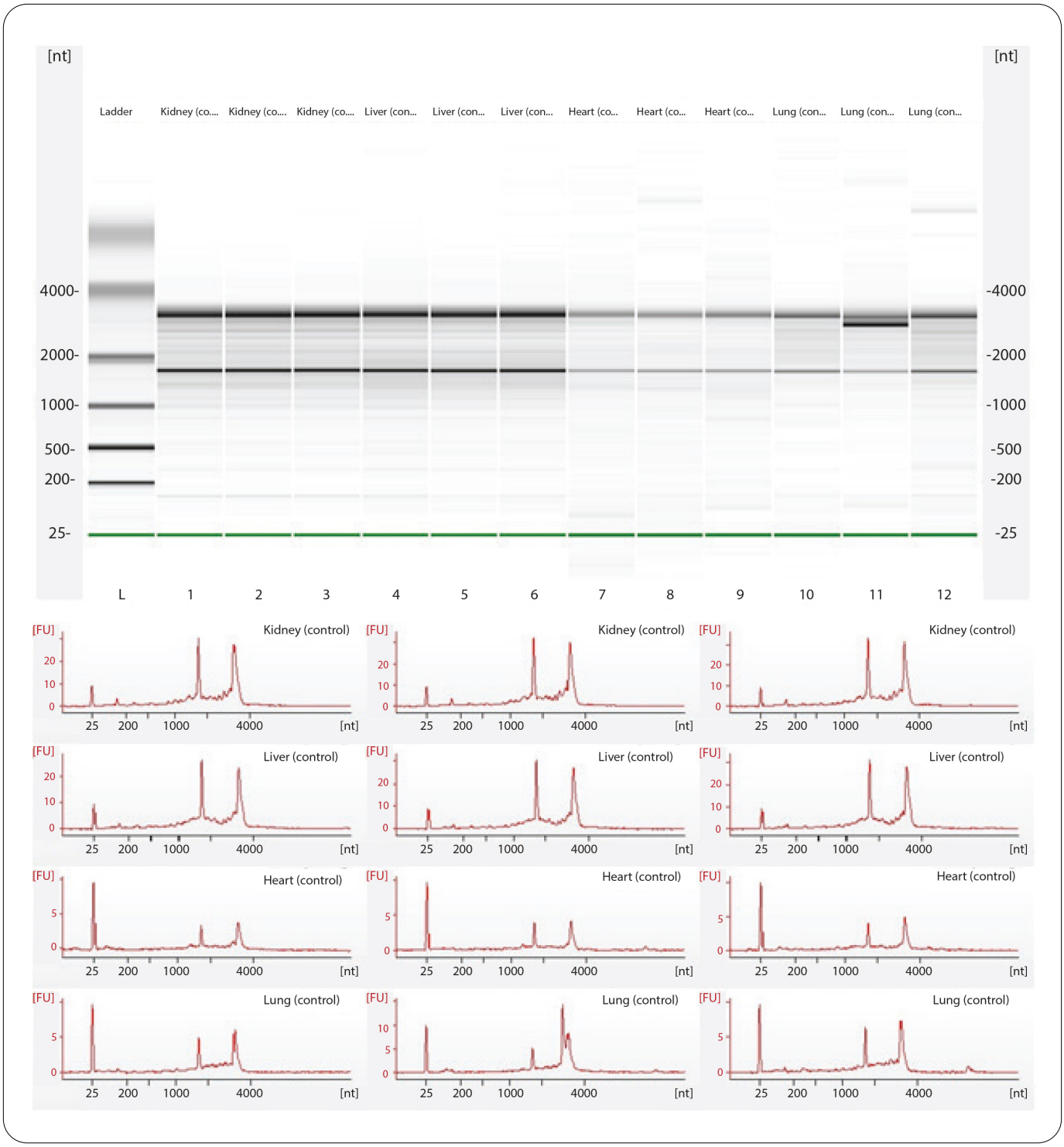


Figure 1: RNA extracted by cryomilling on a liquid nitrogen cooled mortar pestle and purified using a commercially available tissue RNA extraction kit. RNA was analyzed on an automated electrophoresis system.

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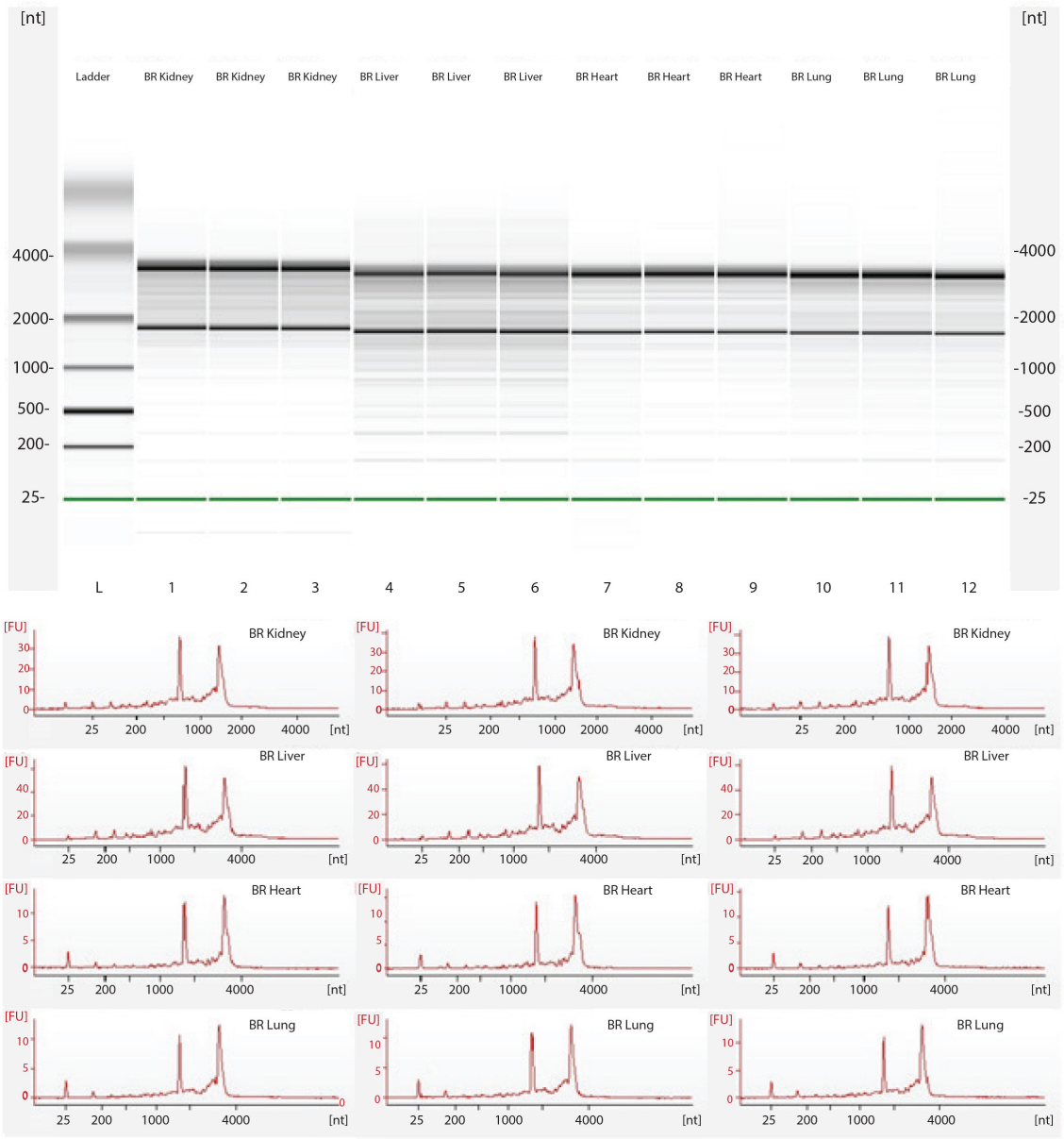

Figure 2: RNA extracted by bead milling on the Omni Bead Ruptor 12 and purified using a commercially available tissue RNA extraction kit. RNA was analyzed using an automated electrophoresis system.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

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