BECKMAN COULTER
Life Sciences

Biomek Automated Genomic Sample Prep Accelerates Research

Biomek i-Series Automated Beckman Coulter Agencourt RNAdvance Tissue Kit

Introduction

The Agencourt RNAdvance Tissue Kit uses Beckman Coulter's patented Agencourt SPRI paramagnetic bead-based technology to isolate total RNA (microRNA and mRNA). After tissue lysis, RNA is immobilized onto the magnetic beads, facilitating RNA separation using a magnetic field (Figure 1). This magnetic separation makes the kit amenable to automation, as it eliminates the need for vacuum filtration or centrifugation. In addition, the protocol can be performed in both 96-well and single tube formats, providing the flexibility in workflow design. In this technical note, we demonstrate automated performance of RNAdvance Tissue Kit on the Biomek i5 Multichannel 96 Genomics Workstation.

When compared to manual operation, the Agencourt RNAdvance Tissue Kit automated on Biomek platform provides:

- Reduced hands-on-time and increased throughput
- Reduction in pipetting errors
- Reduction of cost by using low reagent volumes
- Standardized workflow for improved results
- Quick implementation with demonstrated methods
- Knowledgeable support for reagents, automation and methods all from single vendor

Spotlight: Biomek i5 Multichannel 96 Genomics Workstation

System features deliver reliability and efficiency to increase user confidence and walk-away time, compared to manual operation.

- 300uL or 1200uL Multichannel head with 1-300uL and 1-1200uL pipetting capability
- Enhanced Selective Tip pipetting to transfer custom array of samples
- Independent 360˚ rotating gripper with offset fingers
- High deck capacity provided by 25 positions and separate locations for trash
- Orbital Shakers, peltiers and 96 channel Tip washing for controlling sample processing
- Optional Enclosure to isolate the samples, if desired

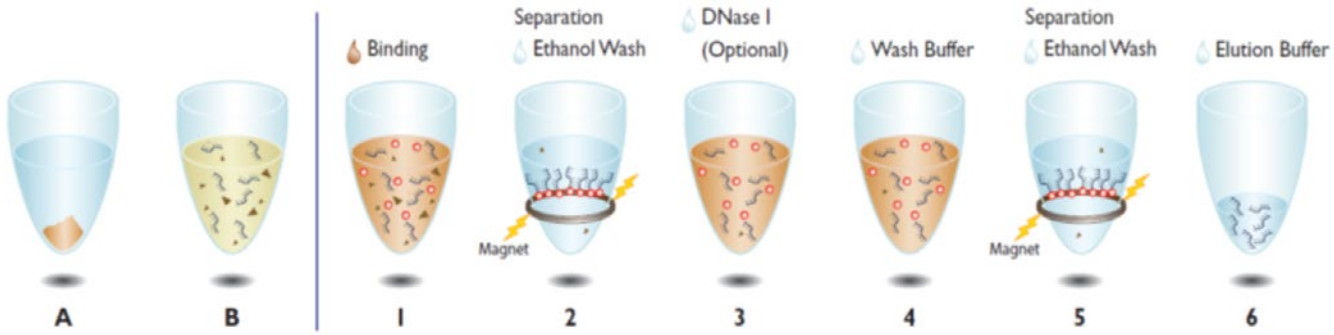

Figure 1. Beckman Coulter Agencourt RNAdvance Tissue Kit protocol

Major Process Description	With DNAse treatment	Without DNAse treatment
Reagent and instrument preparation*	15 min	15 min
Agencourt RNAdvance Tissue method	1 hr 36 min	2 hrs 16 min
Total	**1 hr 51 min**	**2 hrs 31 min**

*: Timing does not include reagent thawing, dissection, tissue lysis and homogenization

Table 1. Estimated run times for Agencourt RNAdvance Tissue Kit on the Biomek i5 Multichannel 96 Genomics Workstation, 1-96 samples

Figure 2. Biomek Method Launcher provides an easy interface to start the method

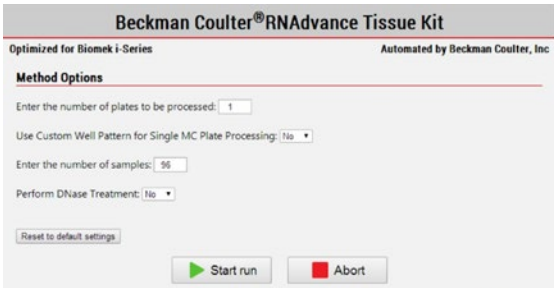

Figure 3. Biomek Method Options Selector indicate sample number and processing options

Automated Method

Biomek i5 Multichannel 96 Genomics Workstation was able to extract RNA from 96 samples in less than 2 hours (Table 1). The use of Biomek Method Launcher simplifies the method implementation and reduces the introduction of errors during method setup.

1. Biomek Method Launcher (BML)

BML is a secure interface for selecting methods without affecting method integrity. It allows the users to remotely monitor the progress of the run. The manual control options provide the opportunity to interfere if needed.

2. Method Options Selector (MOS)

MOS enables selection of plate processing and sample number options to maximize flexibility, adaptability and the ease of method execution.

3. Guided Labware Setup (GLS)

GLS is generated based on options selected in the MOS, and provides the user specific text and graphical setup instructions with reagent volume calculation and step by step instructions to prepare reagents.

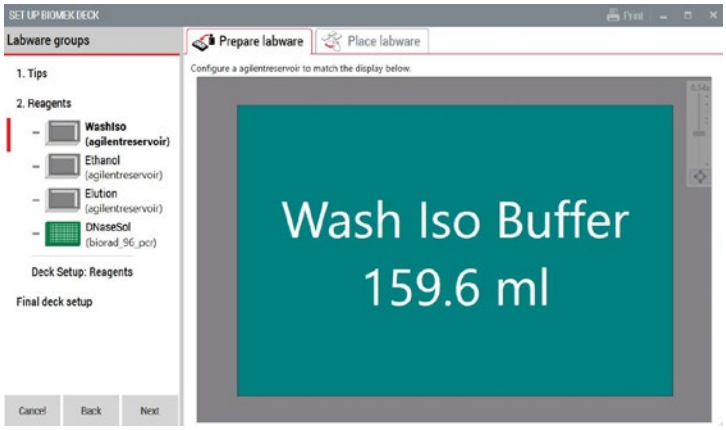

Figure 4. Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup

Experimental Design

Approximately 10 mg of tissues each were excised from frozen mouse kidney (3 replicates), liver (2 replicates) and brain (2 replicates) tissues. RNA was extracted using both manual and automated RNAdvance Tissue kit protocol. The quantity and the quality of the RNA samples were assessed using NanoDrop 2000 TM (Thermo Fisher Scientific), Quant-iT™, RiboGreen® RNA Assay Kit (Thermo Fisher Scientific), Agilent Bioanalyzer 2100 (Agilent RNA 6000 Nano Kit) and qRT-PCR (KAPA SYBR® Fast One-Step qRT-PCR Master Mix kit (2x), Actin B intron flanking transcript, reaction done in triplicates).

Results

High quality RNA is required for many downstream applications. To gain a complete understanding of the RNA extracted by the automated vs. manual protocols, we assessed quality of RNA samples using multiple methods.

To estimate nucleic acid purity, NanoDrop calculates the ratio of the absorbance by the nucleic acid to the absorbance of the contaminants. Both manual and automated samples yielded good A260/A280 ratios (1.8–2.2) and acceptable A260/A230 ratios (>1.7 in general; Table 2).

Quant-iT RiboGreen RNA Assay uses an RNA-specific dye for RNA quantification. The RNA yield of our automated method was comparable with that of the manual extractions (Table 2).

The RNA Integrity Numbers (RIN) calculated by Agilent 2100 Bioanalyzer accounts for not only the ratio of 28S and 18S rRNAs but also the electrophoretic trace of RNA, providing a robust estimate of RNA quality. Both manual and automated methods produced excellent RIN scores (Figure 5). Within tissue variation of RIN score was less for automated samples (Figure 5, Kidney: SD manual 0.7, SD automated 0.5; brain: SD manual 0.9, SD automated 0.1). RIN scores varied based on the tissue type for both manual and automated protocols suggesting the necessity of tissue specific lysis and homogenization protocols (Figure 5).

To demonstrate the usability of extracted RNA in downstream applications, we carried out qRT-PCR. RNA extracted by both methods amplified in the range of C_t 15-20, indicating the superior quality of RNA (Figure 6).

Method	Tissue	NanoDrop		RiboGreen	
		260/280	260/230	Conc. (ng/uL)	Yield (ng)
Manual	Kidney	2.13	1.67	72.257	2890.29
Manual	Kidney	2.13	1.45	59.842	2393.70
Manual	Kidney	2.16	1.45	63.450	2537.96
Manual	Liver	2.05	0.42	18.652	746.10
Manual	Liver	2.04	0.56	102.726	4109.02
Manual	Brain	2.01	0.72	3.5350	141.40
Manual	Brain	1.93	0.84	29.803	1192.11
Automated	Kidney	2.03	1.72	43.130	1725.20
Automated	Kidney	2.09	1.69	38.925	1557.01
Automated	Kidney	2.1	1.58	40.384	1615.36
Automated	Liver	2.17	1.86	62.386	2495.44
Automated	Liver	2.17	1.95	73.161	2926.45
Automated	Brain	2.01	1.24	20.149	805.97
Automated	Brain	1.98	1.35	14.329	573.17

Table 2. The concentrated, yield and the purity of RNA as indicated by NanoDrop 2000 and Quant-iT™ RiboGreen® RNA Assay Kit.

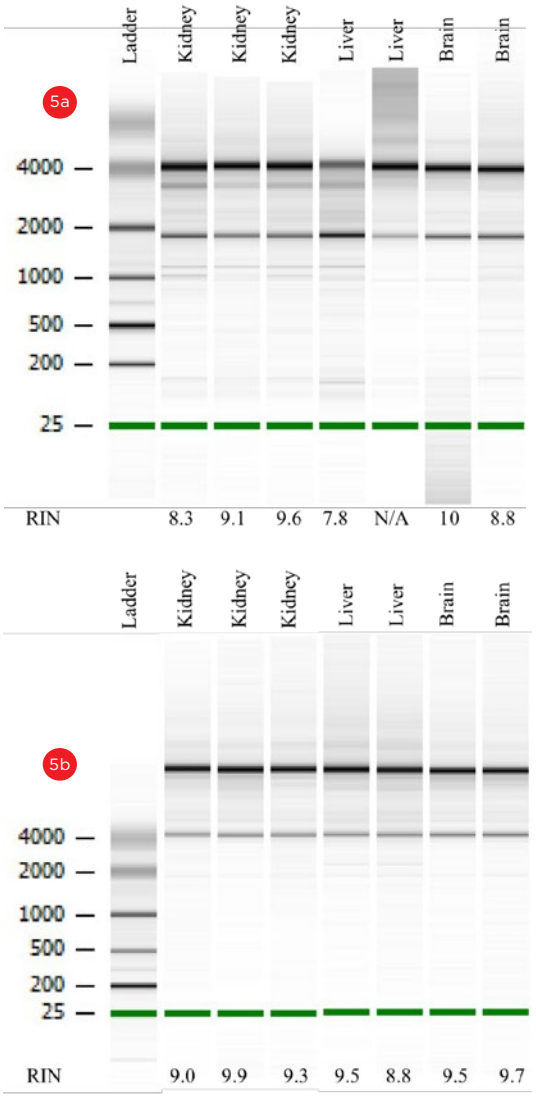

Figure 5. Manual (a) and automated (b) mouse RNA samples were analyzed on Agilent Bioanalyzer 2100.

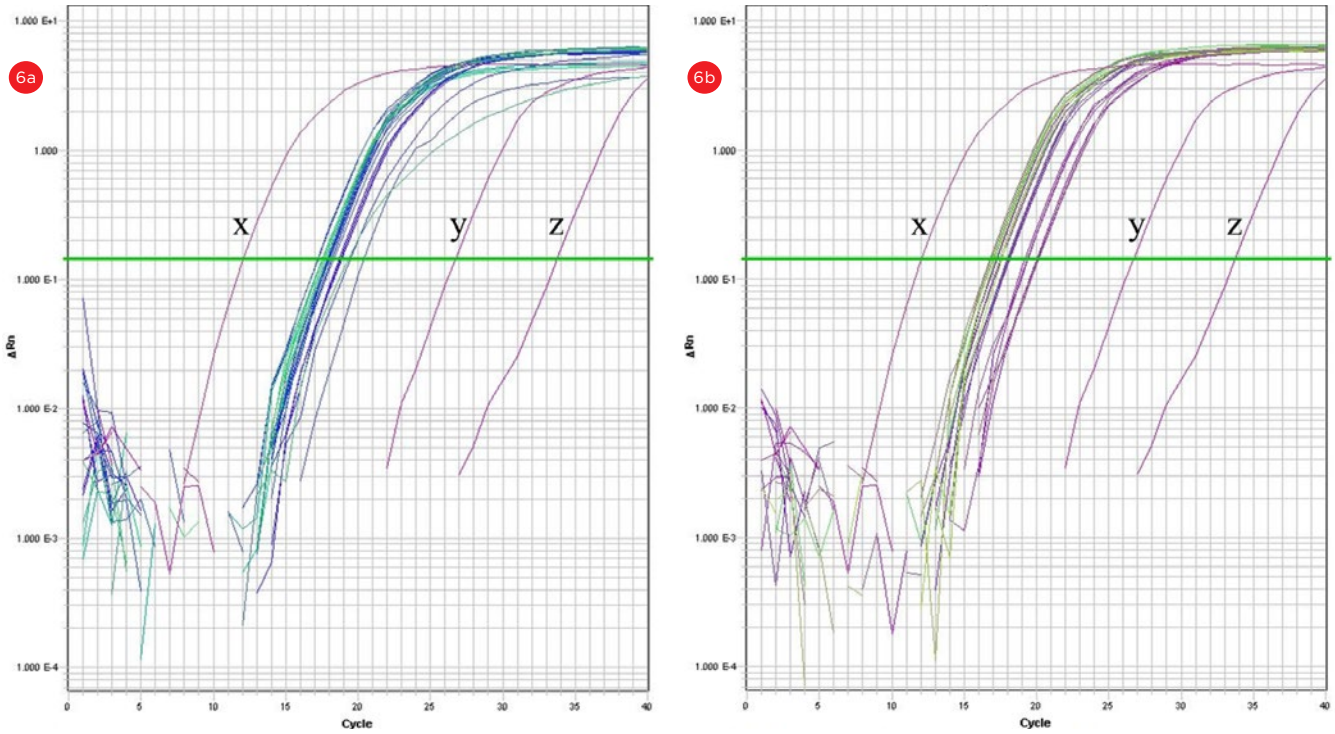

Figure 6. qRT-PCR amplification plots (cycle number vs. florescence) corresponding to manual (a) and automated (b) RNA templates. RNA template concentration 50 ng/uL; X: positive control 200 ng/uL; y: no RT control; z: no template control

Summary

We've demonstrated - automation of Agencourt RNAdvance Tissue Kit on the Biomek i5 Multichannel 96 Genomics Workstation. The quality and quantity of RNA extracted by the automated protocol was comparable to manually extracted RNA. Automation enabled quick and efficient extraction of high quality RNA with low within tissue variation. Not all tissue types behave the same for extraction of nucleic acids. For this reason, the automated protocol like the manual can be easily modified to provide optimal conditions (e.g. Lysis and homogenization) per tissues type.

Data obtained during development

Biomek i-Series Automated Workstations are not intended or validated for use in the diagnosis of disease or other conditions.

For Beckman Coulter's worldwide office locations and phone numbers, please visit "Contact Us" at beckman.com

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