Biomek i-Series Automated Beckman Coulter Agencourt RNAdvance Cell

Introduction

The Agencourt RNAdvance Cell V2 Total RNA extraction kit uses Agencourt's patented SPRI® paramagnetic bead-based technology to isolate total RNA from 200 to 50,000 cultured eukaryotic cells, including cell lines and primary cells. Following cell lysis, RNA is immobilized onto the magnetic beads, to enable RNA separation using a magnetic field (Figure 1). Once bound to the magnetic beads, the RNA can be treated with DNase and the contaminants rinsed away using a simple washing procedure before eluting RNA from the magnetic beads. This magnetic separation makes the kit amenable to automation, as it eliminates the need for vacuum filtration or centrifugation. The purified RNA is eluted using nuclease-free water. Here we demonstrate automated performance of Agencourt RNAdvance Cell Kit on the Biomek i5 Mutichannel Genomics Workstation and Biomek i7 Hybrid Genomics Workstation.

The Agencourt RNAdvance cell V2 Kit automated on Biomek platform provides:

- Reduced hands-on-time and increased throughput compared to the manual operation
- Reduction in pipetting errors compared to the manual operation
- Standardized workflow for improved results
- Quick implementation with ready-to-implement methods
- Knowledgeable support for reagents, automation and methods all a from a 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 the manual operation:

- 300 uL or 1200uL Multichannel head with 1-300 uL and 1-1200 uL 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 sample processing control

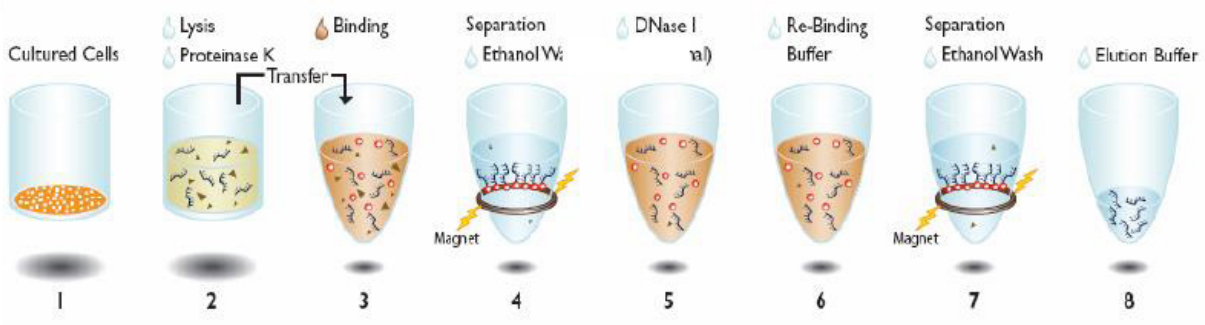

Figure 1. Beckman Coulter Agencourt® RNAdvance Cell V2 Kit protocol

Automated method

Automation provides increased efficiency, reducing the hands on time, compared to the manual operation(Table 1). The use of Biomek Method Launcher simplifies the method implementation and reduces the introduction of errors during method setup.

Process	Time (96 samples; i5 MC)	Time (96 samples; i7 hybrid)
Instrument setup*	30 min	30 min
Method run	2 hrs 41 mins	2 hrs 35 mins
Total	3 hrs 11 mins	3 hrs 5 mins

Table 1. Estimated run times for Agencourt RNAdvance Cell Kit on the Biomek i5 Mutichannel workstation, 1-96 samples*: Timing does not include reagent thawing and homogenization

1. Biomek Method Launcher (BML)

BML is a secure interface for executing 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.

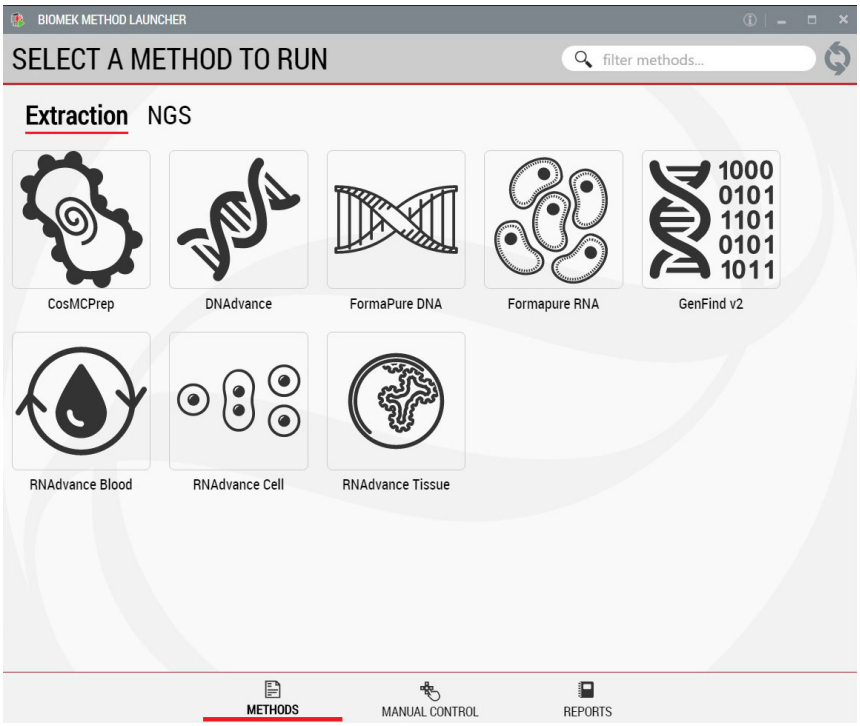

Figure 2. Biomek Method Launcher provides an easy interface to start the method

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. To reduce the time of manual setup, the method provides options to aliquot reagents into processing plates, from the reservoirs.

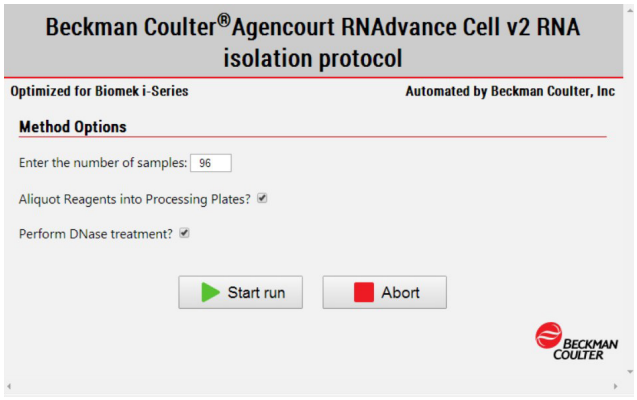

Figure 3. Biomek Method Options Selector indicates sample number and processing options

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.

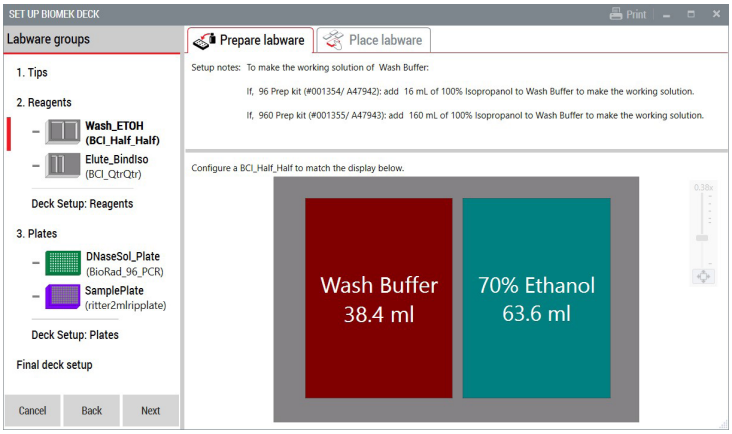

Figure 4. Guided Labware Setup indicates reagent volumes and guides the user for correct deck setup

Experimental design

HCT116 cells were grown in 10% FBS and basal CHO media and counted using Vi-CELL cell counter. The automated extraction protocol was run using 2 replicates, each containing 50,000 HCT116 cells. To compare with the automation, the extraction was also carried out manually (2 replicates, each containing 50,000 HCT116 cells).

The quantity and the quality of the RNA samples were assessed using NanoDrop 2000 ™ (Thermo Fisher Scientific), Agilent TapeStation (HS RNA screen tape) and qRT-PCR (KAPA SYBR® Fast One-Step qRT-PCR Master Mix kit (2x), beta-actin intron flanking transcript, reaction done in duplicates with a negative control and a positive control).

Results

The automated protocol yielded acceptable A260/A280 ratios (1.8–2.2; Table 2). Both manual and automated methods produced good RINe scores (RINe >9.0; 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 Ct 15-20, indicating the superior quality of RNA. No amplification was observed in the negative control (Figure 6).

Sample	A260/280	Total concentration (ng/ µL)
HCT116: Automated replicate 1	2.19	313.3
HCT116: Automated replicate 2	2.19	365.0
HCT116: Manual replicate 1	2.04	291.84
HCT116: Manual replicate 2	2.03	347.12

Table 2: RNA quantity determined by NanoDrop 2000 ™ (Thermo Fisher Scientific)

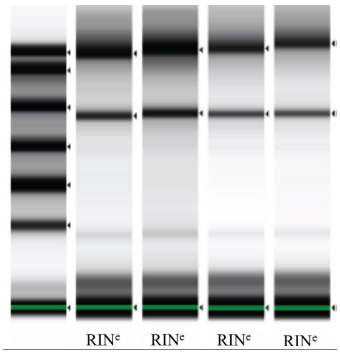

Figure 5. RNA samples were analyzed on Agilent TapeStation. Lane 1: ladder, lanes 2-3: automated protocol; lanes 4-5: manual protocol

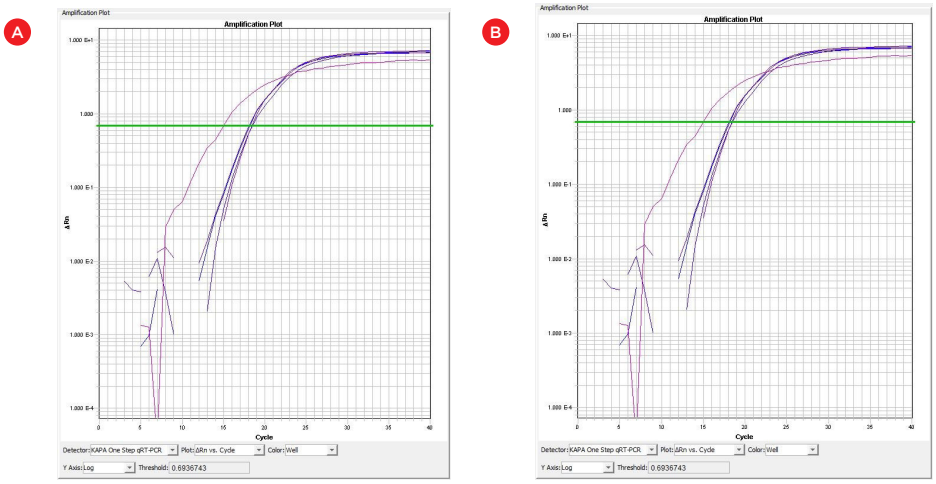

Figure 6. qRT-PCR amplification plots (cycle number vs. florescence) corresponding to automated (a) and manual (b) RNA templates. RNA template concentration 50 ng/uL; X: positive control 200 ng/uL. No amplification in negative control

Summary

We demonstrated the automation of Agencourt RNAdvance Cell V2 Kit on the Biomek i series workstations. The RNA extracted by the automated protocol can be used for downstream applications such as qPCR. Automation enabled quick and efficient extraction. The Biomek Method Launcher provides a user friendly interface to run the method, to decrease setup errors.

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