APPLICATION NOTE

Automating RNA Extraction from Cell Culture and Mammalian Tissue Samples Using Accessible Liquid Handling Technology

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Establishing automation for RNA extraction processes is usually associated with high entry barriers, requiring substantial financial investments, highly trained staff , and huge initial time effort. Once implemented, the economical utilization of these resources is often limited for reasons like uneven demand, e.g. related to project-specific sample income peaks. For these reasons, many laboratories struggle to find the best compromise between the throughput of fully automated extraction solutions and the flexibility of manual processing. In this proof-of-principle study, we present a practical semi-automated solution, combining the advantages of both worlds. The apricot® S3 automated pipettor (SPT Labtech) presents an affordable and easy-to-use liquid handling system, accommodating three deck positions and heads for 8- to 384-channel pipetting. Combined with the EchoLUTION™ extraction technology (BioEcho), RNA from cell or tissue lysates can be extracted in plate formats in unprecedented speed and ease.

Introduction

The integration of liquid handling and automation technologies in biological laboratories offers numerous advantages, revolutionizing the way research and diagnostic tasks are performed. Automation streamlines workflows, allowing for high-throughput processing of samples, and ultimately increasing productivity, reproducibility and safety. Especially in disciplines involving transcriptome analysis, these advantages can have a significant impact by minimizing confounding variables introduced by sample handling, which may bias final datasets and the reliability of analytic results. One of the first crucial steps in such studies involves the extraction of RNA, for example from cells or tissues.

The EchoLUTION technology

The EchoLUTION technology offers a novel approach to nucleic acid extraction, streamlining the purification process into a single step. This method bypasses the need for nucleic acids to interact with the purification matrix, resulting in high purity and yield. It reduces processing time and costs, with an emphasis on sustainability through lower plastic usage and less hazardous substances. Operating under aqueous conditions, the EchoLUTION-extracted nucleic acids are free of inhibitors, making them ideal for sensitive downstream applications. In the following we present the results of a semi-automated extraction of RNA from cells and different tissue types.

Materials and Methods

Liquid handling

Pipetting steps were automated with the apricot S3 (SPT Labtech, Table 1 and Figure 1). The liquid handling instrument offers automated 96 and 384 channel pipetting, suited to low or high-throughput use.

Table 1. SPT Labtech technology and product specifications

apricot S3 liquid handling instrument	
Technology	96 and 384 channel automated pipetting instrument using air displacement technology
Capacity	3 plate positions
Pipetting volumes	1–500 µL (96–500 head)
Equipment/features	Automated split level plate elevators, interchangeable pipetting core, capable of 8, 16, 24, 96 and 384 channel operation, EZ-Load tip technology combined with fully automated X, Y and Z drives, intuitive interface through a Microsoft® Surface Pro Tablet, and seamless integration with API support
Dimensions	457 mm × 406 mm × 584 mm

RNA extraction from cultured cells

The EchoLUTION Cell Culture RNA Kit was developed for RNA extraction from human and animal cultured cells (Table 2). The kit is much faster than established kits on the market, with 20 minutes total time required for a 96-well plate.

For this experiment, we extracted RNA from nine dry cell pellets in a 96-well plate (HEK 293, 5 × 10^5 cells per sample). To mimic a full 96-well plate extraction, the rest of the wells were processed without sample.

For total RNA extraction, we followed the instructions in the kit's user manual, while automating all liquid handling steps using the apricot S3 (Figure 2): 100 µL of

Figure 1. apricot S3 liquid handling instrument

Lysis Buffer Cell RNA were added into each well. After incubation for 5 minutes at 40°C and 1,400 rpm on a heat shaker (Thermomixer® C, Eppendorf®), 15 µL of Clearing Solution were aliquoted into each well using the apricot S3. After brief mixing and centrifugation, 5 µL DNA removal master mix were aliquoted to each well and incubated for 10 minutes at 40°C and 300 rpm. After incubation, 100 µL lysate was transferred to a prepared Purification Plate using the apricot S3 and purified by centrifugation.

Table 2. EchoLUTION Cell Culture RNA Kit product specifications

EchoLUTION Cell Culture RNA Kit	
Extraction technology	EchoLUTION single-step purification
Sample material	Up to 2 × 10^6 cells (fresh or stored)
Purified nucleic acid	Total RNA including small RNA
Expected yield	Up to 30 µg depending on starting material
Output volume	Up to 100 µL

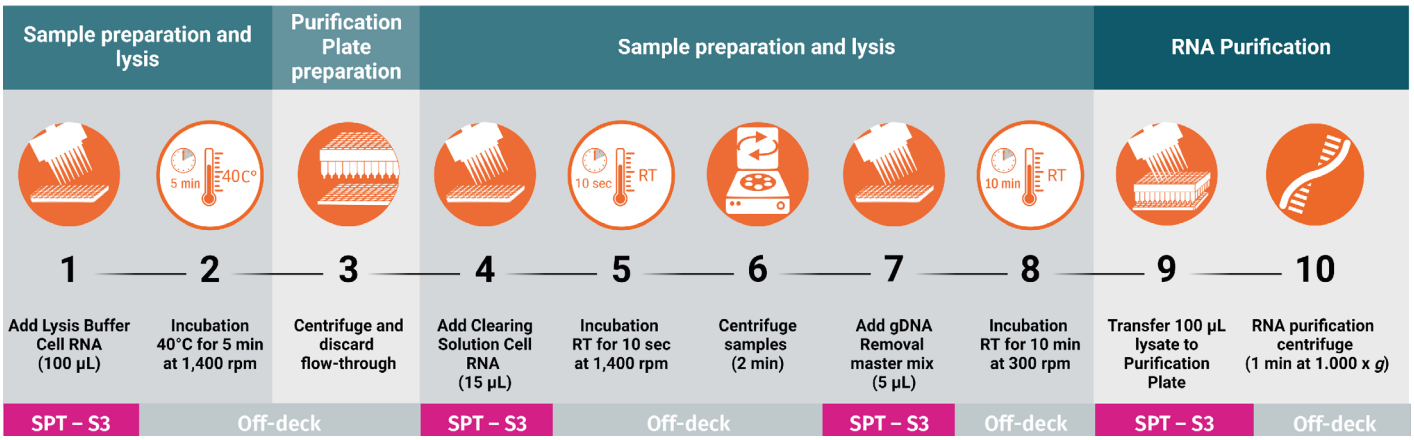

Figure 2. Automation of the EchoLUTION Cell Culture RNA Kit (1 × 96 samples). *All steps that have been automated using the apricot S3 automated pipettor (SPT Labtech) are indicated in pink. Manual, "off-deck" steps are indicated in grey.*

RNA extraction from tissue samples

The EchoLUTION Tissue RNA Kit enables the isolation of RNA from various tissue types (Table 3). With an ultra-fast lysis, it allows the RNA from 96 samples to be extracted within 35 minutes.

As part of this study, we extracted RNA quadruplicates from fresh frozen rat brain (10 mg), lung (10 mg) and liver tissue samples (5 mg). To mimic a full 96-well plate extraction, the rest of the wells were processed without sample.

We extracted RNA following the kit's user manual. All pipetting steps were done using the apricot S3 (Figure 3):

Table 3. EchoLUTION Tissue RNA Kit product specifications

EchoLUTION Tissue RNA Kit	
Extraction technology	EchoLUTION single-step purification
Sample material	Up to 10 mg of fresh-frozen or stabilized mammalian tissue (e.g., liver, muscle, spleen, lung, and nervous tissue)
Purified nucleic acid	Total RNA including small RNA
Expected yield	Up to 14 µg depending on tissue type
Output volume	Up to 80 µL

After adding 150 µL Lysis Buffer Tissue RNA, we homogenized the tissue samples with one 4 mm Steel Bead each in a 96-well plate utilizing a bead beater (MM300, Retsch) for 4 × 30 seconds at 30 Hz. After short centrifugation (Rotina 380, Hettich) and incubation at 40 °C on a heat shaker (Thermomixer C, Eppendorf), 22 µL clearing solution were aliquoted to each well. After mixing and centrifugation, 7 µL of DNase reaction mix were added and incubated for 10 minutes while shaking at 300 rpm. Finally, the samples were subjected to single step purification by transferring 80 µL lysate to the prepared Purification Plate and purified by centrifugation.

RNA quantification and quality measurements

As a parameter for RNA quality, RNA integrity (RIN) was determined using a TapeStation® 4150 (Agilent Technologies®) with Agilent RNA ScreenTape according to the manufacturer's manual.

RNA concentrations were determined fluorometrically using the Qubit™ RNA BR Assay (Thermo Fisher Scientific) according to protocol. Eluate volumes were measured manually by pipetting to determine the total yield.

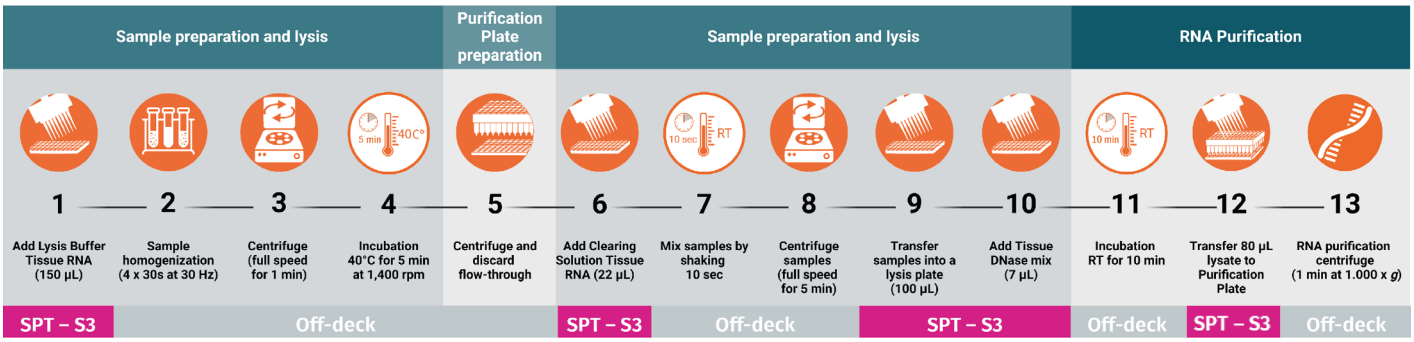

Figure 3. Automation of the EchoLUTION Tissue RNA Kit (1 × 96 samples). *All steps that have been automated using the apricot S3 automated pipettor (SPT Labtech) are indicated in pink. Manual, "off-deck" steps are indicated in grey.*

Results

Cell culture RNA extraction

Extracted RNA was analyzed on TapeStation. The RINe data (Figure 4) indicate no degradation and high integrity levels for the RNA obtained with the EchoLUTION Cell Culture RNA Kit.

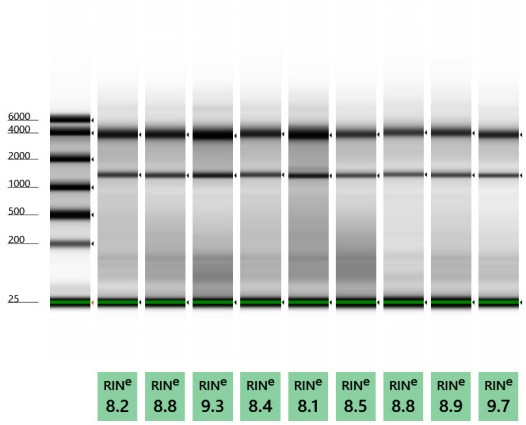

Figure 4. Quality evaluation of the RNA extracted from HEK 293 cells (5 × 10⁵ cells each) with the EchoLUTION Cell Culture RNA Kit; liquid handling automated using the apricot S3

RNA concentrations and yields are summarized in Figure 5 and are well within the expected range of results obtained by manual processing (data not shown).

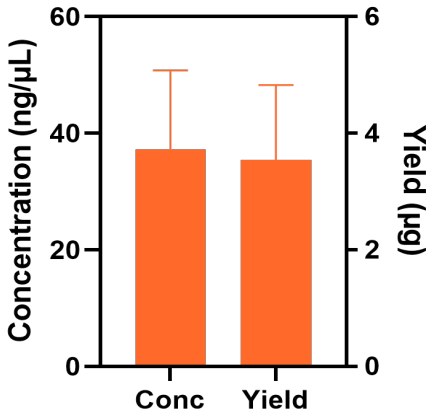

Figure 5. Concentration and yield of the RNA isolated from HEK293 cells (5 × 10⁵ cells per sample; n = 9) with EchoLUTION Cell Culture RNA Kit; liquid handling automated using the apricot S3

Tissue RNA extraction

Again, extracted RNA was evaluated using a TapeStation. The RINe data (Figure 6) indicate high integrity levels for the RNA obtained with the EchoLUTION Tissue RNA Kit, especially for liver and brain tissue samples.

RNA concentrations and yields are summarized in Figure 7 and are, again, similar to previous results when manually processing the kit (data not shown).

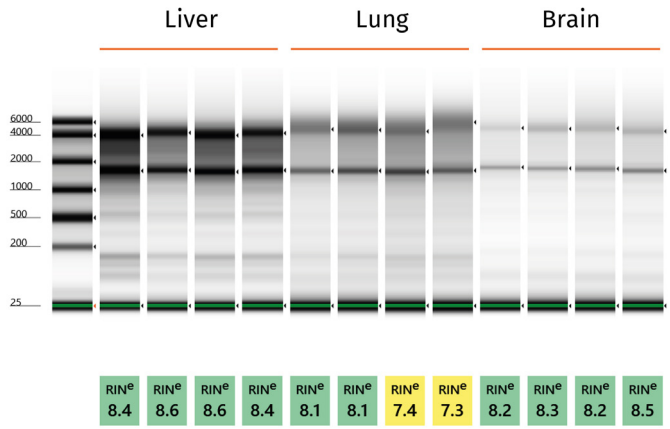

Figure 6. Quality evaluation of the RNA extracted from liver, lung, and brain tissues with the EchoLUTION Tissue RNA Kit; liquid handling automated using the apricot S3

Consumables and time required

Both extraction workflows, whether manual or automated, require very few steps and hence utilize less consumables than conventional kits on the market. In this proof of principle study, we could show that with the EchoLUTION Cell Culture RNA Kit a 96-well plate can be processed on the apricot S3 liquid handling instrument with less than two tips per sample (Table 4). For the EchoLUTION Tissue RNA Kit, less than three tips per sample were required in the same setup.

The time required for the total extraction workflows, including lysis, was very similar to manual processing using an 8-channel pipette. We could, however, save three (Cell Culture RNA) and six (Tissue RNA) more minutes, respectively, when processing a 96-well plate with the apricot S3 (data not shown).

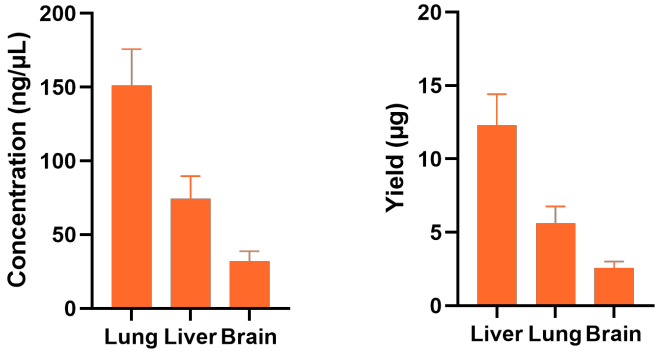

Figure 7. Concentrations and yields of the RNA isolated from rat lung (10 mg input), liver (5 mg), and brain (10 mg) using the EchoLUTION Tissue RNA Kit; liquid handling automated using the apricot S3 . n = 4 for each sample type

Table 4. Consumables required for automated RNA extraction workflows with the EchoLUTION Cell Culture RNA Kit and EchoLUTION Tissue RNA Kit using the apricot S3. *All tips from SPT Labtech, stated quantities apply for one 96-well plate.*

Extraction kit	Tips used	# of tips
EchoLUTION Cell Culture RNA Kit	Load strip tips 550 µL (8 × 12 tips)	24
	EZ-load strip tips 400 µL (96 tips)	96
EchoLUTION Tissue RNA Kit	Load strip tips 550 µL (8 × 12 tips)	24
	EZ-load strip tips 400 µL (96 tips)	192

Conclusion

In this proof-of-principle study for semi-automated nucleic acid extraction, we were able to demonstrate an easy-to-set up and economically attractive solution that combines the advantages of automation with the flexibility of a manual workflow. Pairing the EchoLUTION extraction technology with the apricot S3 automated pipettor, RNA could be extracted from cell or tissue lysates in plate formats with unmatched speed and ease.

Our findings highlight several key advantages:

- **Simple usability:** System setup and programming protocols and labware is intuitive, ensuring a smooth workflow without advanced training necessary.
- **Robust pipetting:** The tip loading system demonstrated to be very reliable; pipetting without any tip blockages, even for demanding tissue lysates.
- **High flexibility and reproducibility:** The combined system offers high flexibility in handling various sample types while maintaining reproducibility in results.
- **High throughput at small footprint:** All hardware required for extraction can be flexibly accommodated on a lab bench and enables extractions of multiple plates per hour.

Ordering information

Product	Reactions	Product no.
EchoLUTION Tissue RNA Kit	2 × 96 8 × 96	011-115-002 011-115-008
EchoLUTION Cell Culture RNA Kit	2 × 96 8 × 96	011-114-002 011-114-008

About SPT Labtech

SPT Labtech makes products that transform the way scientists work. For nearly two decades, our expert scientists, engineers, and business innovators have created innovative solutions for liquid handling, sample preparation and management that help accelerate research and make a real difference to human health. We work collaboratively with our customers, building trusted relationships that enable us to deliver exceptional, personalized experiences designed for real world challenges in the lab. For more information, please visit: www.sptlabtech.com

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