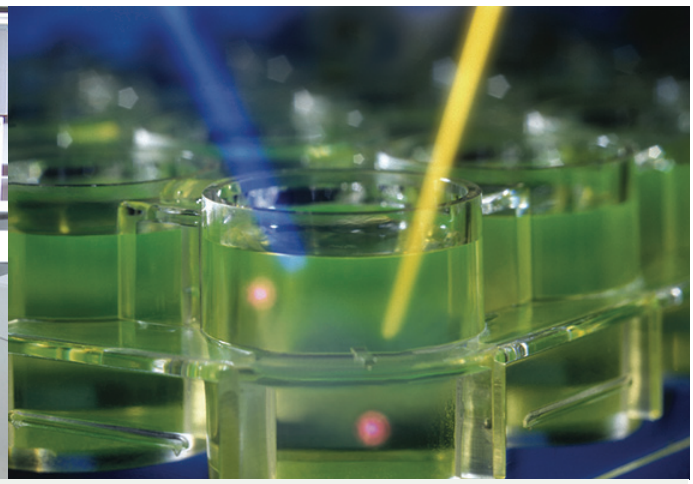

How to quickly and reliably obtain highly pure RNA

Reliable extraction of RNA from cells and tissue with high yield and purity using the NucleoSpin® 96 RNA kit on a Freedom EVO® platform

Introduction

Purification of high quality RNA from large numbers of samples represents a serious bottleneck in sample processing for general screening projects and gene expression studies. Furthermore, reliability, process control, sample integrity and the avoidance of cross-contamination are major issues for the purification of RNA.

Tecan and MACHEREY-NAGEL have joined forces to provide a flexible automated solution for the purification of RNA from cells and tissue samples for research use only, not for use in clinical diagnostics. MACHEREY-NAGEL's NucleoSpin 96 RNA kit for fast extraction of high purity RNA is suitable for a broad range of downstream applications, such as RT-PCR or cDNA synthesis. The purification method is based on vacuum filtration using silica membrane technology, in combination with on-column DNase treatment and suitable binding, wash and elution buffers, and can be fully automated on the Freedom EVO platform.

The system can be set up in a matter of minutes, gaining considerable walkaway time and relieving staff from tedious, repetitive jobs to perform more highly skilled tasks. This automated solution reduces common risks such as cross-contamination between samples and carry-over of chemicals and solvents, while reducing manual errors and maximizing reproducibility. In addition, full sample tracking further improves overall process security.

The high purity of the extracted RNA is demonstrated by an average $A_{260/280}$ ratio of 2.1, in addition to excellent PCR performance. High yields of up to 11 µg RNA are obtained from 10^6 HeLa cells. Full automation of the RNA purification process on a Freedom EVO workstation streamlines laboratory workflows and provides reliable, fast extraction of highly pure RNA.

Materials and Methods

Equipment

The Freedom EVO liquid handling workstation can be equipped with a 2-, 4- or 8-channel Liquid Handling (LiHa) Arm, with disposable tip adapters and a low level disposable tip ejection option to reduce cross-contamination. A Robotic Manipulator (RoMa) Arm assembles the Te-VacS™ vacuum module, which can accommodate either MACHEREY-NAGEL´s 96-well binding plates or 8-well binding strips. The system also includes a Te-Shake™ module for fast, optimal mixing of samples and buffers (Figure 1).

Sample numbers	Up to 96 samples, in multiples of 8 or 96
Batch time	1 h 20 min for 96 samples
Equipment Tecan	• Freedom EVO 100 platform, 8-channel Liquid Handling Arm configured for disposable tips, 1,000 µl syringes, Robotic Manipulator Arm, stainless steel deck and safety panel set • Te-Shake • Microplate, trough, tube and disposable tip carriers • Wash station with waste disposal • Disposable tips (filtered) 1000 µl • 25 ml and 100 ml troughs • Freedom EVOware® Standard software
Equipment MACHEREY-NAGEL	• NucleoSpin 96 RNA kit • Column Holder A (required for 8-well strips only)

Table 1 Overview of equipment for high and medium throughput RNA purification

Automated workflow

Up to 30 mg of tissue or 2 x 10^6 cultured cells are harvested and processed in a microplate format. Samples are placed onto the platform and the RNA is purified without any user intervention. The fully automated RNA purification procedure includes direct lysis of cells grown in 96-well plates, if not already using homogenized samples, binding of RNA to silica membranes, on-column DNase treatment, stringent wash steps and final elution of the purified RNA.

The configuration and scripting of the Tecan workstation have been optimized to minimize the risk of cross-contamination and maximize the yield and quality of nucleic acids.

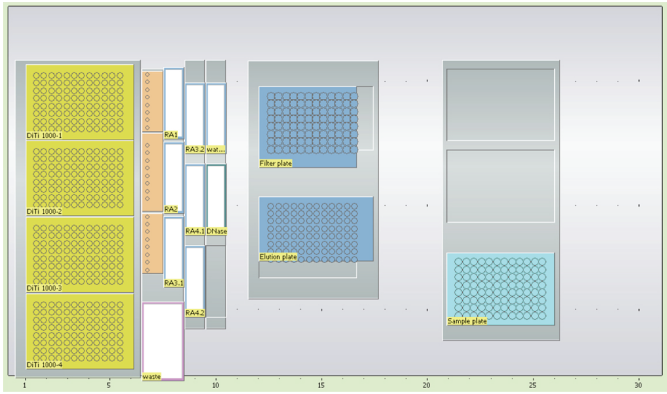

Figure 1 Optimized Freedom EVO worktable layout
Worktable layout for RNA extraction, including Te-VacS and Te-Shake modules and use of the RoMa and LiHa Arms

Results

Automation of the NucleoSpin 96 RNA kit on the Freedom EVO sample preparation workstation allows fast, convenient and reliable purification of RNA from a variety of sources, including tissues and cultured cells. The automated method produces isolated RNA of excellent purity with no substantial degradation (Figures 2 and 3), and the yield is consistently high (Figure 4). The complete automated purification of 96 cell culture samples takes 1h 20 mins.

Purity

The purity of RNA purified with the MACHEREY-NAGEL NucleoSpin 96 RNA kit is excellent. With mouse tissue samples, average $A_{260/280}$ ratios of 2.1 were obtained (Figure 2), and the eluted RNA is highly pure (Figure 3) and free of contaminants, allowing a broad range of downstream applications such as PCR and real-time PCR.

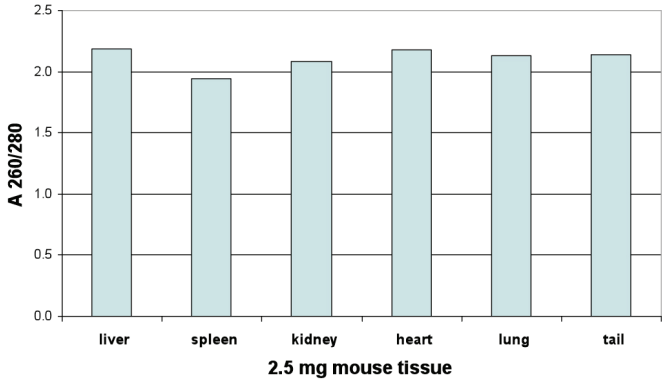

Figure 2 Excellent purity of RNA isolated from different tissue samples
RNA was purified from 2.5 mg mouse tissue samples. Each bar represents the average $A_{260/280}$ ratio from eight tissue samples.

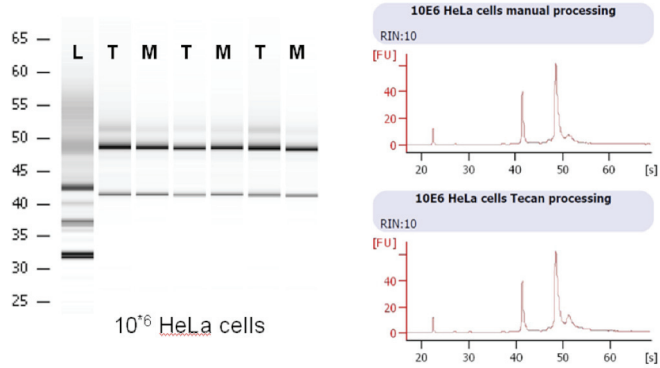

Figure 3 RNA purified from cultured cells shows little degradation
RNA was isolated from 10^6 cultured cell samples with either the manual (M) or automated (T) method. L: DNA ladder. Obtained RIN quality score is 10, indicating the high integrity of purified RNA.

Yield and Reliability

Assay reproducibility and intra-assay variation is shown in Figure 5. RNA from 2.5 mg of mouse liver tissue was purified to perform 96 extractions from identical aliquots, giving an RNA yield of 17.3 µg with a CV of 7.57 %. The data highlights the robustness and consistency of the automated procedure. Figure 4 shows typical yields obtained from cultured HeLa cells.

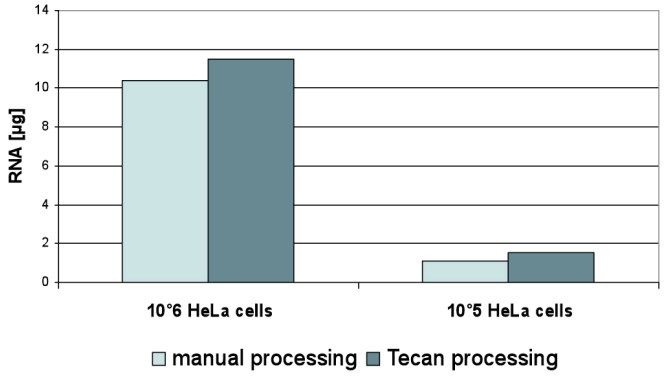

Figure 4 High yield of RNA purified from cultured cells
RNA was isolated from 10^6 and 10^5 HeLa cells with either the manual or the automated method. Each bar represents the average from eight cell culture samples.

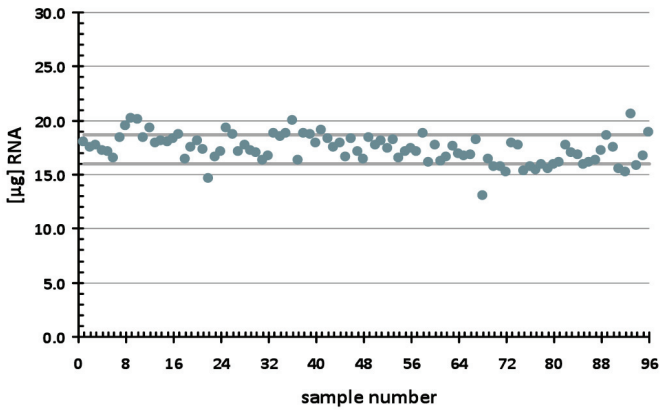

Figure 5 Highly reproducible RNA purification process
RNA was purified from 2.5 mg of mouse liver tissue samples. An average RNA yield of 17.3 µg was obtained. The CV of 7.57 % demonstrates the high reproducibility of the purification process.

Downstream applications and cross-contamination

The purified RNA is suitable for a broad range of downstream applications. An RT-PCR-based method was chosen to demonstrate the quality of the RNA purified by the automated process. An aliquot of the purified RNA was amplified by RT-PCR targeting a housekeeping gene. A dilution series of samples was prepared, as shown in Figure 6, and the desired RT-PCR product was amplified in all samples proportionally to its RNA starting concentration.

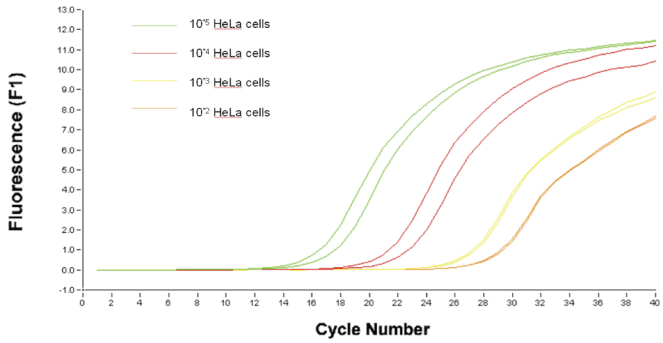

Figure 6 Real-time RT-PCR amplification of eluted RNA
Aliquots of the purified RNA were amplified by real-time RT-PCR targeting a GAPDH gene fragment. All samples were amplified.

To demonstrate the absence of cross-contamination, tissue samples, plus PBS buffer as negative controls, were arranged in a square-well block in a checkerboard pattern. RNA isolation of both positive and negative samples was performed using the automated NucleoSpin 96 RNA kit protocol, and aliquots (4 µl) of the eluates were subjected to real-time RT-PCR. The results are illustrated in Figure 7, with no amplification detected in the negative samples, indicating the absence of cross-contamination.

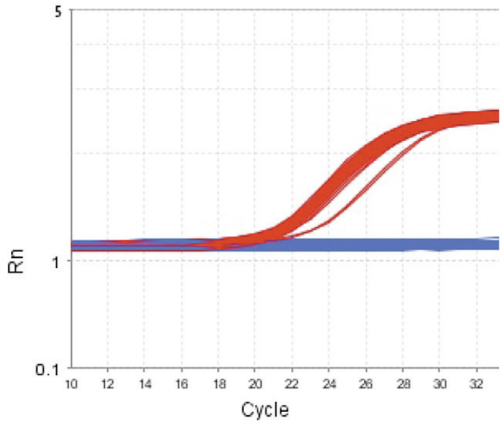

Figure 7 Cross-contamination analysis
4 µl of RNA eluate was amplified by RT-PCR (ABI, 7500 Real-Time PCR System, beta-Actin, 200 bp fragment, FAM labeled probe, 40 cycles). Specific RT-PCR products were amplified only from the wells which were filled with tissue samples for RNA isolation (red curves), no specific RT-PCR product was obtained from the wells filled with PBS buffer (blue lines). No cross-contamination was observed.

Conclusion

Automation of the NucleoSpin 96 RNA kit on a Tecan Freedom EVO sample preparation workstation enables fast, reliable extraction of RNA from cell culture and tissue samples in a truely walkaway manner, consistently generating high quality RNA. For highest flexibility, or to meet changing laboratory needs, the Freedom EVO sample preparation workstation can be equipped with a number of extension modules, including an absorbance reader, storage modules and cooling devices.

Talk to your local Tecan representative to customize the Freedom EVO workstation to meet your specific laboratory requirements.

Acknowledgements

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Further Application Notes

Updated list at www.tecan.com\machereynagel

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