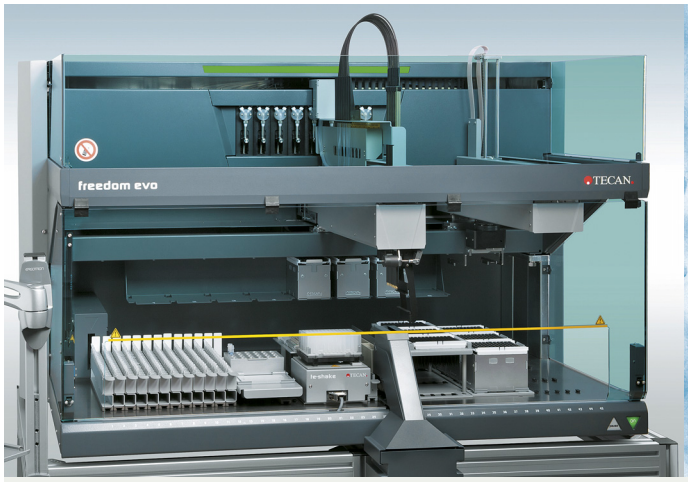

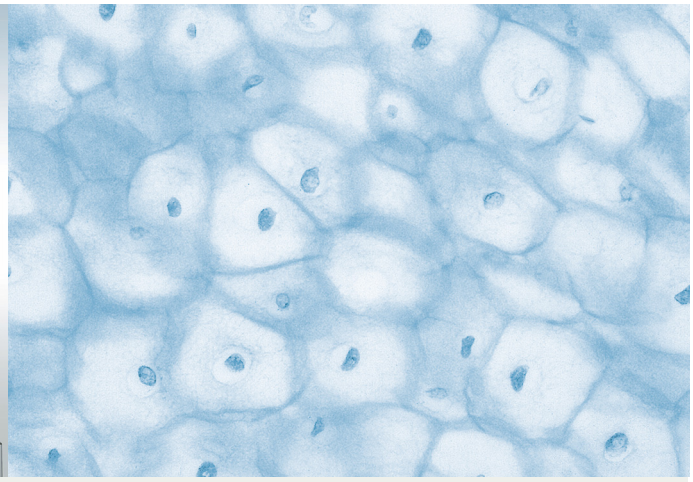

Reliable and flexible purification of RNA

Extraction of RNA from cells and tissue with high yield and purity with the NucleoMag® 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. MACHEREY-NAGEL's NucleoMag 96 RNA kit for fast extraction of high purity RNA is suitable for a broad range of downstream applications, such as real-time PCR (RT-qPCR), cDNA synthesis or next generation sequencing. The purification method is magnetic bead-based, keeping the workflow very flexible with regard to scalability (amount of starting material) and sample numbers, 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 manual errors, cross-contamination between samples and carry-over of chemicals and solvents, while 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 9 µg RNA are obtained from 5 x 10^5 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 transfers the samples on to and off the MACHEREY-NAGEL NucleoMag SEP Magnetic Separator. The system also includes a Te-Shake™ module for fast, optimal mixing of samples and buffers (Figure 1).

Sample numbers	Up to 96 samples
Batch time	2 h 50 min for 96 samples
Equipment Tecan	• Freedom EVO 100 platform, 8-channel LiHa Arm configured for disposable tips, 1000 µl syringes, RoMa 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	• NucleoMag® 96 RNA kit • Square-well blocks • NucleoMag SEP Magnetic Separator

Table 1 Overview of equipment for high throughput RNA purification

Automated workflow

Up to 20 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 magnetic beads, on-bead 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.

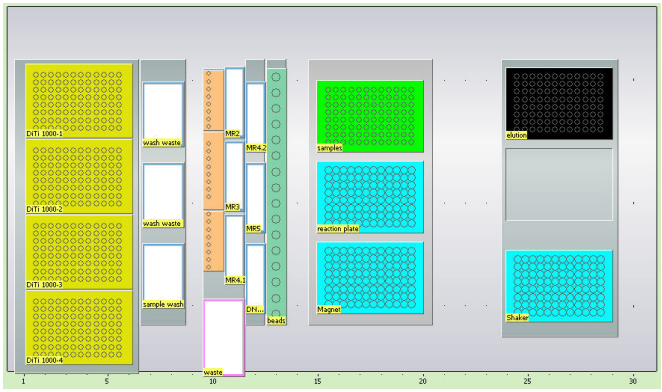

Figure 1 Optimized Freedom EVO worktable layout for RNA purification using both RoMa and LiHa Arms, and including a Te-Shake module and a NucleoMag SEP Magnetic Separator

Results

Automation of the NucleoMag 96 RNA kit on the Freedom EVO sample preparation workstation allows fast, convenient and reliable purification of RNA from a variety of sources, including tissue and cultured cells. The automated method isolates RNA of excellent purity without any substantial degradation (Figures 2 and 3), and the yield is consistently high (Figures 4 and 5). The complete automated purification of 96 cell culture samples takes 2h 50 mins.

Purity

The purity of RNA purified with the MACHEREY-NAGEL NucleoMag 96 RNA kit is excellent. Using mouse tissue and cultured cell samples, average $A_{260/280}$ ratios of 2.1 were obtained (Figure 2). Furthermore, the eluted RNA is of high integrity (Figure 3) and free of contaminants, allowing a broad range of downstream applications, such as RT-qPCR.

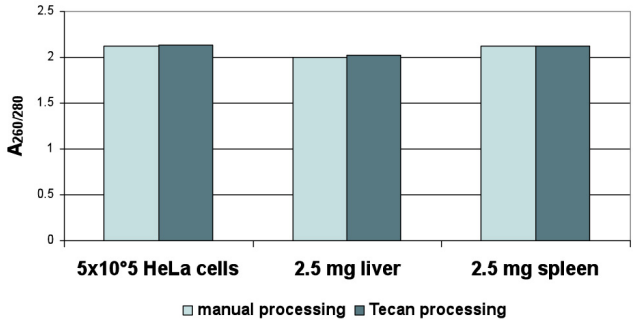

Figure 2 Excellent RNA purity obtained from liver, spleen and tissue samples and cultured cells. RNA was purified from 5 x 10^5 HeLa cells or 2.5 mg mouse tissue samples. Each bar represents the average $A_{260/280}$ ratio of eight samples.

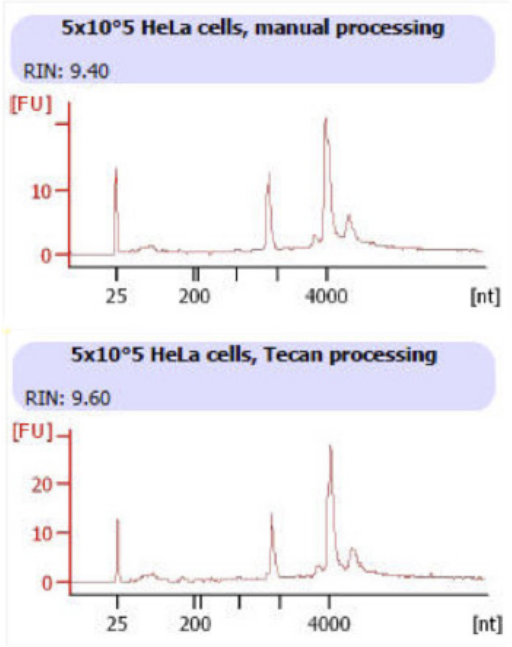

Figure 3 RNA purified from cultured cells has high integrity. RNA was isolated from 5 x 10^5 cultured cell samples with either the manual or automated method. The RIN quality score obtained is between 9.4 and 9.6, indicating the high integrity of purified RNA.

Yield and reliability

Figure 4 shows typical yields obtained from cultured HeLa cells and mouse tissue. Assay reproducibility and intra-assay variation is shown in Figure 5. RNA was isolated from 96 identical aliquots of 5 x 10^5 HeLa cells, resulting in an RNA yield of 7.3 µg with a CV of 4.1 %. The data highlights the robustness and consistency of the automated procedure.

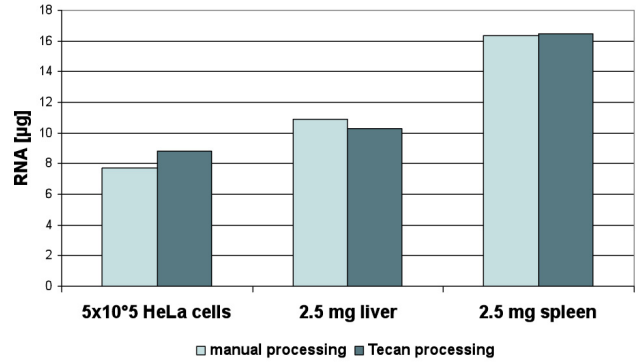

Figure 4 High yield of RNA purified from cultured cells. RNA was isolated from 5 x 10^5 HeLa cells or mouse tissue samples with either the manual or the automated method. Each bar represents the average from eight samples.

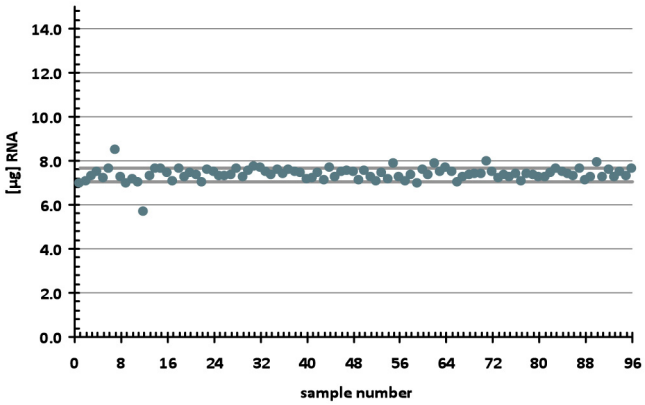

Figure 5 Highly reproducible RNA purification process. RNA was purified from 5 x 10^5 HeLa cell samples. An average RNA yield of 7.3 µg was obtained. The CV of 4.1 % demonstrates the high reproducibility of the purification process.

Downstream applications and cross-contamination

The purified RNA is suitable for a broad range of downstream applications. A RT-qPCR-based method was chosen to demonstrate the quality of the RNA purified by the automated process. Aliquots of the purified RNA from different tissue and cultured cell samples were amplified by RT-qPCR targeting a housekeeping gene. The desired RT-qPCR product was amplified in all samples (Figure 6).

To demonstrate the absence of cross-contamination, cultured cell samples, plus PBS buffer as a negative control, were arranged in a square-well block in a checkerboard pattern. RNA isolation of both positive and negative samples was performed using the automated NucleoMag 96 RNA kit protocol. For quantitative analysis, aliquots (4 µl) of the eluates were subjected to RT-qPCR.

The results show that no amplification was detected in the negative control samples, indicating the absence of any cross-contamination (Figure 7).

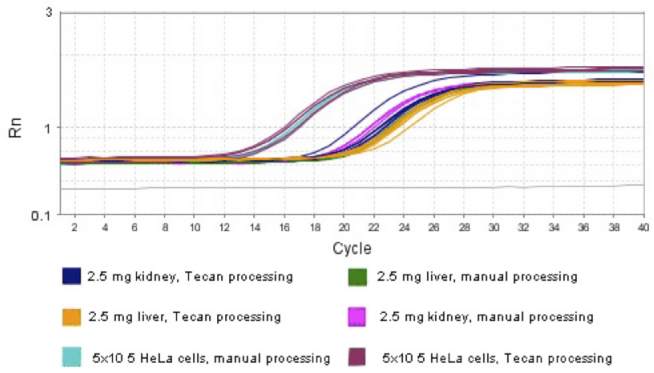

Figure 6 RT-qPCR amplification of eluted RNA. Aliquots of the purified RNA were amplified by RT-qPCR targeting a beta-actin gene fragment. All samples were amplified.

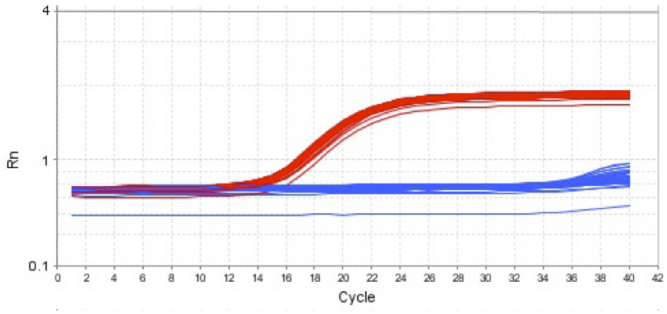

Figure 7 Cross-contamination analysis. 4 µl of RNA eluate was amplified by RT-qPCR (ABI, 7500 Real-Time PCR System, beta-actin, 130 bp fragment, 40 cycles). Specific RT-qPCR products were amplified only from the wells which were filled with cultured cell samples for RNA isolation (red curves), no specific RT-qPCR product was obtained from the wells filled with PBS buffer (blue lines). No cross-contamination was observed.

Conclusion

Automation of the NucleoMag 96 RNA kit on a Tecan Freedom EVO sample preparation workstation enables fast and reliable RNA extraction from cell culture and tissue samples in a truly 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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MACHEREY-NAGEL GmbH & Co. KG
Neumann-Neander-Strasse 6, 52355 Düren, Germany

Further Application Notes

Updated list at www.tecan.com/machereynagel

Austria +43 62 46 89 33 **Belgium** +32 15 42 13 19 **China** +86 21 2206 3206 **Denmark** +45 70 23 44 50 **France** +33 4 72 76 04 80 **Germany** +49 79 51 94 170
Italy +39 02 92 44 790 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 174 **Singapore** +65 644 41 886 **Spain** +34 93 490 01 74 **Sweden** +46 31 75 44 000
Switzerland +41 44 922 89 22 **UK** +44 118 9300 300 **USA** +1 919 361 5200 **Other countries** +41 44 922 8125

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