Applications

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Automated RNA purification in 96-well plate and 8-well strip format from human cells or animal tissue using the MACHEREY-NAGEL NucleoSpin® 8/96 RNA kits on the ep*Motion*® 5075 from Eppendorf

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Abstract

In this application note we describe the integration of the MACHEREY-NAGEL NucleoSpin® 8/96 RNA kits into the ep*Motion*® 5075 VAC automated pipetting system. The NucleoSpin 8/96 RNA kits are based on a well proven vacuum filtration based bind-wash-elute procedure. Protocols for the ep*Motion* 5075 VAC are available for medium throughput using the flexible 8-well strip based purification kit or for high throughput using the 96-well plate based kit. The use of NucleoSpin 8/96 RNA kits on the ep*Motion* 5075 VAC allows the isolation of total RNA from cells or tissue. Application data for RNA isolation from mouse or pork tissue and human cells are presented. RNA quality was tested by analysis on the Agilent 2100 Bioanalyzer and in qRT-PCR experiments.

Introduction

The isolation of total RNA from cells and tissue is a prerequisite for analysis of gene expression or gene expression profiling by qRT-PCR methods. RNA has to be extracted with high yield and purity. In addition RNA has to be virtually DNA-free and of high structural integrity. The demands of an automated procedure on RNA isolation are robustness with respect to different sample materials, flexibility regarding sample throughput, speed and easy automation. Most of the drawbacks of conventional RNA isolation can be overcome by state-of-the-art silica membrane purification. Here, we describe the use of the MACHEREY-NAGEL NucleoSpin 8/96 RNA kits for use on the ep*Motion* 5075 VAC automated pipetting system. Using the well proven bind-wash-elute procedure, liquid-liquid extraction with toxic organic solvents or RNA precipitation can be avoided. The NucleoSpin 8/96 RNA kits provide excellent consistency, a high robustness even when using very diverse sample types such as cells and tissues from various organs, and uncompromised RNA yield and quality. In addition, the kits can readily be automated on liquid handling systems. The NucleoSpin RNA procedure is based on sample lysis using chaotropic salt buffer. The lysis buffer deactivates RNase activities and stabilizes RNA. The use of optional pre-filter lysate clearing strips or plates supports the removal of debris obtained from tissue homogenization. Following the binding of RNA on the silica membrane remaining DNA is removed by an on-column DNase digestion step. In subsequent washing steps DNase, proteins and other contaminants are removed. Finally the purified RNA is eluted in water and can be used for further downstream applications. The use of an optimized silica membrane allows RNA isolation from up to 2 x 10^6 cells or 10-30 mg of tissue. Depending on the sample type and amount yields of up to 20-40 µg RNA can be achieved.
The purified RNA is suitable for use in several downstream applications, e.g. real-time RT-PCR. The use of NucleoSpin 8/96 RNA kits on the epMotion 5075 automated pipetting system provides excellent results without the need for extensive programming, optimization and set-up time.

Materials and Methods

Eppendorf ep*Motion* 5075 VAC
Vac Frame 2
Vac Holder
Reservoir 400 mL
Collection Plate Adapter for MN Tube Strips
Channeling Plate
Reservoir Rack with Reagent Reservoirs
MACHEREY-NAGEL NucleoSpin 96 RNA kit
MACHEREY-NAGEL NucleoSpin 8 RNA kit
tissue samples (e.g. mouse organs)
eukaryotic cells (HeLa S3 cells)

Product use limitations and safety information
Please read the MACHEREY-NAGEL NucleoSpin 8 RNA or NucleoSpin 96 RNA manual before performing the method for the first time.

Determination of yield and purity
Yield and purity of RNA were determined using a microplate reader (Biotek, Powerwave™ 200). RNA yield was calculated from A_{260} values. Purity was determined by calculating the $A_{260/280}$ ratio.

Bioanalyzer
Integrity and quality of RNA was analyzed using the Agilent RNA 6000 Nano assay on the Agilent 2100 Bioanalyzer.

Real-time RT-PCR
Real-time RT-PCR was performed with human or mice specific GAPDH primer sets using the Roche LightCycler® instrument with the Sigma SYBR® Green Quantitative RT-PCR kit according to manufacturer´s instructions.

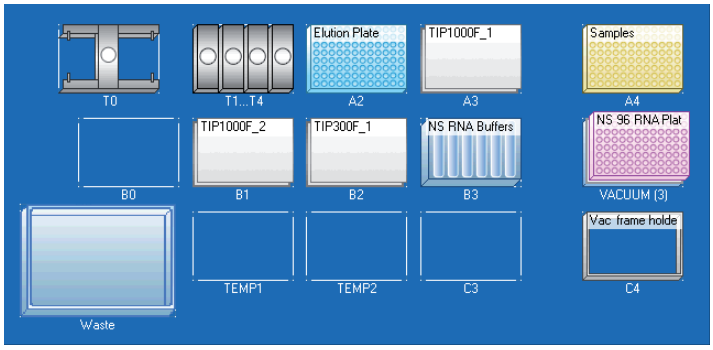

Figure 1: Screenshot from the ep*Motion* Editor showing the setup of the ep*Motion* 5075 VAC worktable for use with the NucleoSpin 96 RNA kit.

Table 1: ep*Motion* 5075 VAC worktable details for NucleoSpin 96 RNA protocol

Position	Labware	Comment
A2	Elution plate (MN_MTP_320)	MTP for elution
A3	epT.I.P.S Motion Filter 1000 µL	
A4	2.1 mL deep-well plate (MN_DWP_2100)	samples
B1	epT.I.P.S Motion Filter 1000 µL	
B2	epT.I.P.S Motion 300 µL	
B3	Reagent Reservoirs Position 1: empty Position 2: Buffer RA4 Position 3: Buffer RA3 Position 4: Buffer RA3 Position 5: Buffer RA2 Position 6: water Position 7: DNase	 100 mL reservoir 100 mL reservoir 100 mL reservoir 100 mL reservoir 30 mL reservoir 30 mL reservoir
Vacuum	NucleoSpin RNA Binding Plate (MN_FP_96_1500) Vacuum Frame 2 Reservoir 400 mL with channeling plate	RNA binding plate (top) collar for vacuum manifold collects waste
C4	Vacuum Frame Holder	Height adapter for vacuum Frame 2
T0	Gripper	
T1	TM 1000-8	1000 µL 8-channel pipetting tool
T2	TM 300-8	300 µL 8-channel pipetting tool

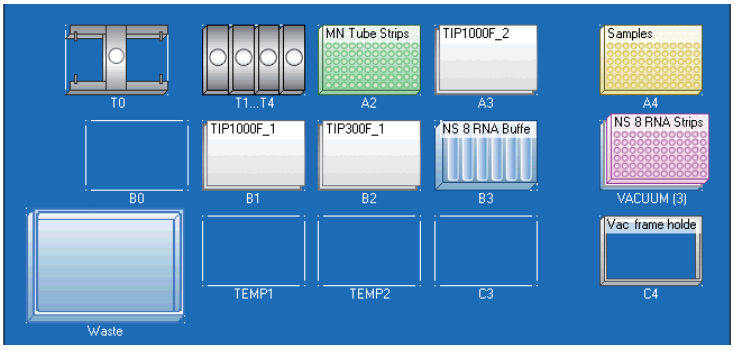

Figure 2: Screenshot from the ep*Motion* Editor showing the setup of the ep*Motion* 5075 VAC worktable for use with the NucleoSpin 8 RNA kit.

Results

Reproducibility of yield and purity of total RNA isolated from pork liver tissue using the NucleoSpin 96 RNA kit
In order to demonstrate the reproducibility of the purification method pork liver was homogenized in lysis buffer RA1 and the crude lysate was distributed in the individual wells of a 96-well deepwell plate. Each well of the deepwell plate represents a tissue sample amount of 10 mg. RNA isolation from tissue started with the addition of RA4 buffer. Purification of RNA from the 96 samples was achieved in 90 min. RNA yield and purity are shown in figure 3. The results are summarized in table 3. Highly reproducible results for yield and purity were obtained.

Table 2: epMotion 5075 VAC worktable details for NucleoSpin 8 RNA protocol

Position	Labware	Comment
A2	MN Tube Strips (MN_TP_1200_48)	elution tubes* (***)
A3	epT.I.P.S Motion 1000 µL	
A4	2.1 mL deep-well plate (MN_DWP_2100)	samples
B1	epT.I.P.S Motion Filter 1000 µL	
B2	epT.I.P.S Motion Filter 300 µL	
B3	Reagent Reservoirs Position 1: empty Position 2: Buffer RA4 Position 3: Buffer RA3 Position 4: empty Position 5: Buffer RA2 Position 6: water Position 7: DNase	 100 mL reservoir 100 mL reservoir 100 mL reservoir 30 mL reservoir 30 mL reservoir
Vacuum	NucleoSpin RNA Binding Strips (MN_FP_8_1400) Vacuum Frame 2 Reservoir 400 mL with channeling plate	RNA binding strips inserted into Column Holder A (top) collar for vacuum manifold collects waste
C4	Vacuum Frame Holder	Height adapter for vacuum Frame 2
T0	Gripper	
T1	TM 1000-8	1000 µL 8-channel pipetting tool
T2	TM 300-8	300 µL 8-channel pipetting tool

*) require Collection Plate Adapter for MN tube strips, see ordering information
**) 8-well strips are inserted into MACHEREY-NAGEL Column Holder A which is part of the Starter Set A, see ordering information
***) 96 well MTP can be used optionally

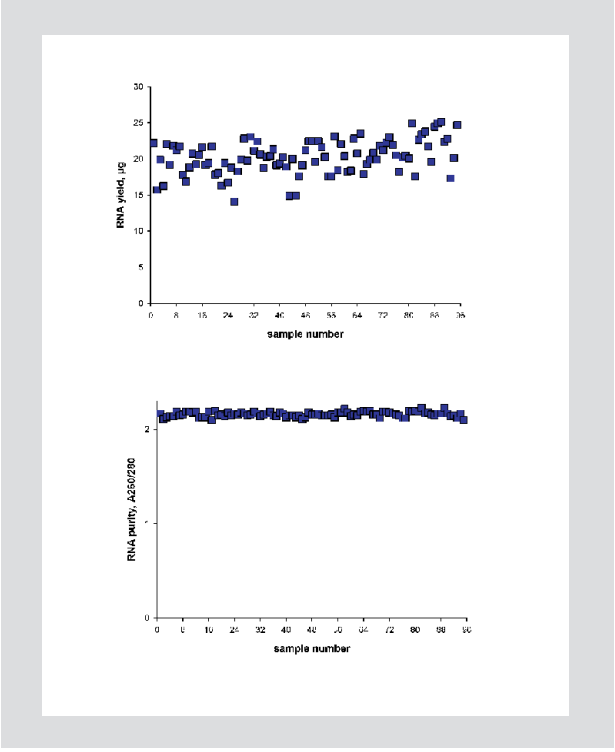

Figure 3: Reproducibility of RNA isolation from pork liver using the NucleoSpin 96 RNA kit.
Total RNA was isolated using NucleoSpin 96 RNA kit as described above. RNA yield and purity were determined spectrophotometrically.

Table 3: Yield and purity of RNA isolated from pork liver tissue using the NucleoSpin 96 RNA kit.

	RNA yield (µg)	RNA purity ($A_{260/280}$)
average yield / purity	20.27	2.16
standard deviation	2.35	0.03
min. yield / purity	14.1	2.23
max. yield / purity	25.1	2.10

Reproducibility of yield and purity of total RNA isolated from HeLa S3 cells using the NucleoSpin 8 RNA kit
For the isolation of RNA from cultured cells a batch culture of HeLa S3 cells grown in culture bottles was distributed into a 96-well deepwell plate. The cell number of each well corresponds to approx. $7.5x10^5$ cells. Cells were pelleted by centrifugation and PBS buffer was discarded. The samples were then processed with the NucleoSpin 8 RNA kit (total number of samples = 48). The deepwell plate with the cell pellet was placed at the indicated position of the ep*Motion* 5075 VAC deck. RNA isolation from cells was started on the ep*Motion* 5075 VAC instrument with the addition of the lysis buffer RA1. RNA yield and purity were determined spectrophotometrically and are shown in Fig 4. The results are summarized in Table 4. Highly reproducible results for yield and purity were obtained.

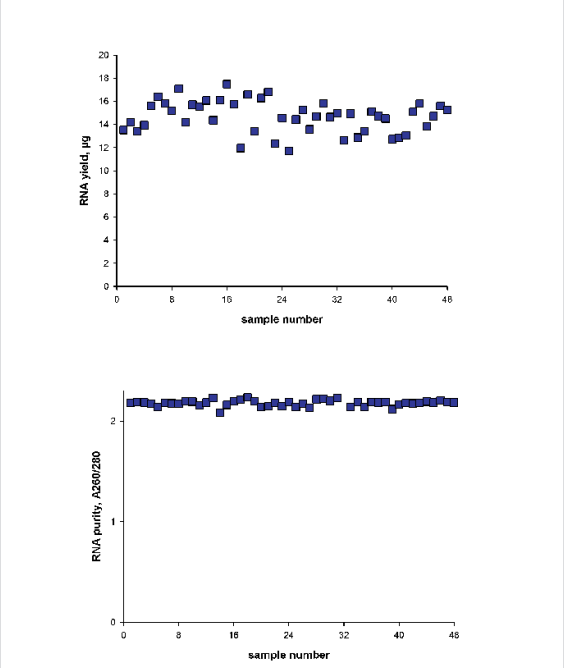

Figure 4: Reproducibility of RNA isolation from HeLa S3 cells using the NucleoSpin 8 RNA kit.
Total RNA was isolated using NucleoSpin 8 RNA kit as described above. RNA yield and purity were determined spectrophotometrically.

Table 4: Yield and purity of RNA isolated from HeLa S3 cells using the NucleoSpin 8 RNA kit.

	RNA yield (µg)	RNA purity ($A_{260/280}$)
average yield / purity	14.68	2.18
standard deviation	1.38	0.04
min. yield / purity	11.73	2.08
max. yield / purity	17.48	2.36

For isolation of total RNA from cells grown in 96-well plates the procedure needs slight adjustment. Due to the volume capacity of the individual wells of the plate the added volume of lysis buffer RA1 is reduced to 130 µL. The volume of the solution RA4 is reduced to 130 µL accordingly.

Quality of RNA and structural integrity
In order to demonstrate quality and structural integrity of the isolated RNA randomly selected purified RNA samples from cells or tissue were analyzed with the Agilent 2100 Bioanalyzer using the RNA 6000 Nano assay. The RNA migrates with sharp bands of 28S and 16S ribosomal RNA. The appearance of the RNA bands and the absence of additional bands in the fast migration area demonstrate the high structural integrity of the RNA. For further analysis the RNA integrity number (RIN) was calculated by the Agilent Bioanalyzer software. High RIN scores confirm the excellent quality of the RNA isolated from tissue. Exemplary profiles of the Bioanalyzer are shown in figure 5, the results for average RIN number and 28S/18S rRNA ratios are shown in table 5.

Table 5: RIN number and rRNA ratio [28S/18S]

	HeLa S3 cells	mouse liver	mouse pancreas
number of samples analyzed	12	6	6
average RIN	9.1	7.8	8.7
average rRNA ratio [28S/18S]	1.9	1.4	1.7

RNA quality and suitability for downstream applications
The isolated total RNA is ready to use and can be directly used in subsequent downstream applications like one or two-step RT-PCR analysis. As an example we demonstrate the use of purified total RNA from mouse liver or pancreas and total RNA from HeLa cells in an one-step real-time RT-PCR system targeting the GAPDH gene. Amplification plots show reproducible threshold cycles (CT values). Furthermore the end-point product analysis does not show any evidence for RT-PCR inhibitors. Amplification plots are shown in figure 6.

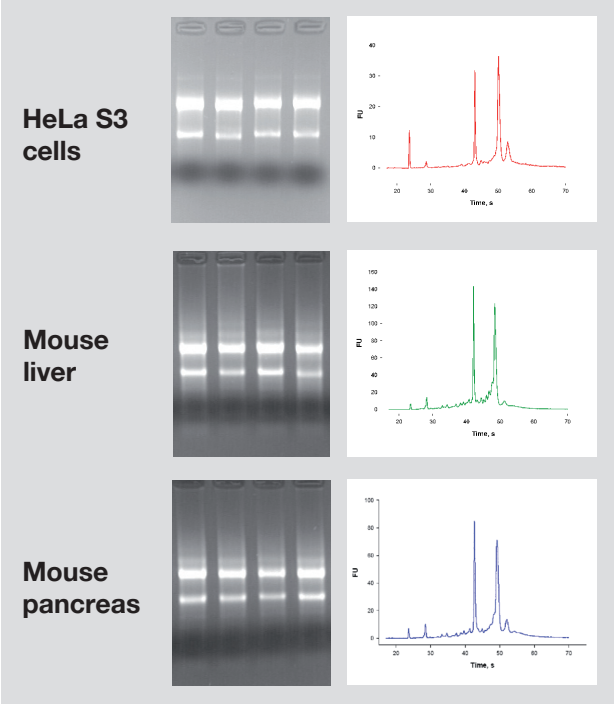

Figure 5: Agilent 2100 Bioanalyzer electropherogramms and agarose gel analysis of RNA isolated with the NucleoSpin 96 RNA kit.
1 µL of purified RNA either from HeLa S3 cells or from mouse liver or mouse pancreas tissue were analyzed on the Agilent Bioanalyzer using the RNA Nano 6000 assay.

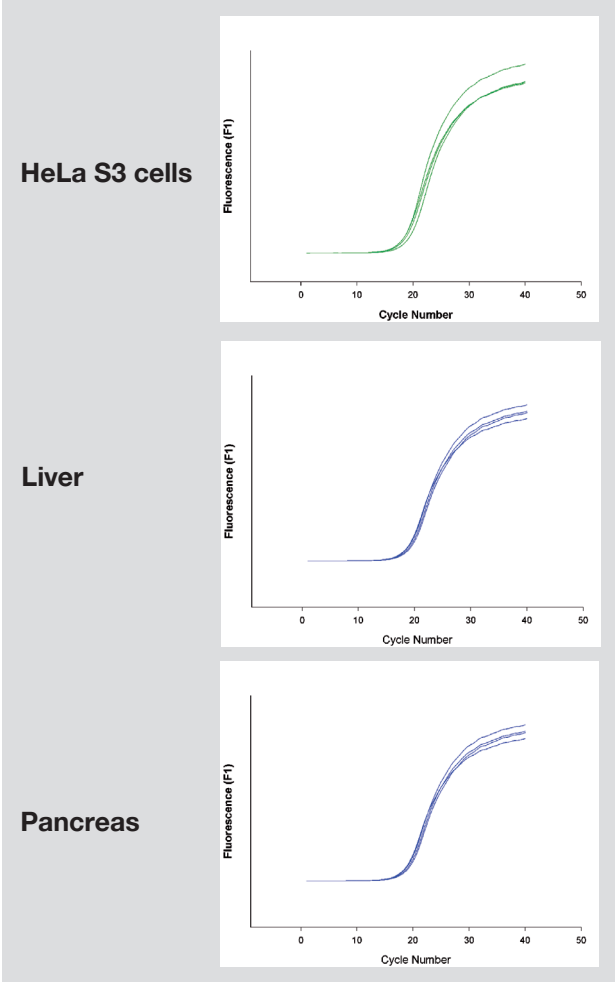

Figure 6: Real time qRT-PCR targeting the GAPDH gene.
All purified total RNA samples from each sample type were amplified with consistent threshold cycles. Average threshold cyles are: HeLa cells 27.06, liver 18.49, pancreas 18.66.

Conclusion

The integration of the MACHEREY-NAGEL NucleoSpin 8 RNA and 96 RNA kits into the ep*Motion* 5075 VAC resulted in a flexible system for automated purification of high quality total RNA from cells and tissue samples. The system can be used either for low to medium throughput using the 8-well strip based NucleoSpin 8 RNA kit or for higher throughput using the 96-well based NucleoSpin 96 RNA kit. The possibility to use additional lysate clearing strips or plates allows the isolation from higher starting amounts of cells or tissue samples. Both kits can be used with the same hardware allowing the user to switch between the two methods according to the requirements in sample throughput. The purified RNA is of excellent quality and, for example, suitable for downstream RT-PCR. In summary, the NucleoSpin technology and the ep*Motion* 5075 VAC automated pipetting system form an attractive and versatile system for the automated isolation of total RNA from different sample materials.

References

Eppendorf

Operating Manual ep*Motion* 5075

Plug´n´Prep Method file available at www.epmotion.com/pnp

MACHEREY-NAGEL

NucleoSpin 8 RNA kit user manual

NucleoSpin 96 RNA kit user manual

Ordering Information Eppendorf

Product	Order no. international	Order no. North America
Collection Plate Adapter	5075 785.030	960002531
Channeling Plate	5075 794.004	960002540
ep*Motion*® 5075 VAC 230 V (vacuum chamber included)	5075 000.164	n/a
ep*Motion*® 5075 VAC 120 V (vacuum chamber included)	n/a	960020014
Dispensing tool TM 1000-8	5280 000.258	960001061
Reservoir Rack	5075 754.002	960002148
Reservoirs 100 mL (10 x 5 reservoirs in bags/case, PCR clean)	0030 126.513	960051017
Reservoirs 30 mL (10 x 5 reservoirs in bags/case, PCR clean)	0030 003.993	960050100

Ordering Information MACHEREY-NAGEL

Product	Order no.
NucleoSpin® 8 RNA kit, 12 x 8 preps	740 698
NucleoSpin 8 RNA kit, 60 x 8 preps	740 698.5
NucleoSpin 96 RNA kit, 2 x 96 preps	740 709.2
NucleoSpin 96 RNA kit, 4 x 96 preps	740 709.4
NucleoSpin 96 RNA kit, 24 x 96 preps	740 709.24
Starter Set A (for NucleoSpin 8 RNA only), 1 set	740 682

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SYBR is a registered trademark of Molecular Probes, Inc.
LightCycler is registered trademark of Roche

MACHEREY-NAGEL

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