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Automated Purification of RNA from Cells or Tissue with the MACHEREY-NAGEL NucleoMag® RNA Kit on the ep*Motion*® 5075

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Abstract

The purification of RNA from cell or tissue samples is routinely performed in a variety of research laboratories with a growing demand for automation. Automated Liquid Handling significantly improves the purification process, especially for potentially hazardous sample materials. The NucleoMag RNA Kit from Macherey-Nagel was adapted to the ep*Motion* 5075t/m. The combination of ep*Motion* 5075t/m and the purification kit, allows walk away purification of RNA from up to 96 cell or tissue samples in less than 100 minutes. High yield and purity, absence of PCR inhibitors and absence of cross-contamination is demonstrated.

Introduction

The procedure of the NucleoMag 96 RNA kit is based on reversible adsorption of nucleic acids to paramagnetic beads under appropriate buffer conditions.
Cells or tissue are lysed with Lysis buffer MR1supplemented with TCEP (Reducing Agent). The contaminants are removed through three washing steps with wash buffers MR3 and MR4.
The purified RNA is eluted and can be used directly as a template for qPCR, next generation sequencing, or any kind of enzymatic reactions.
This application note describes the configuration and preparation of the ep*Motion* 5075m/t to automate this kit.

Materials and Methods

Required Labware
> Eppendorf ep*Motion* 5075t or 5075m
> Dispensing Tool TM 1000-8
> Dispensing Tool TM 300-8
> Gripper
> Reservoir Rack
> Reservoirs 30 mL
> Reservoirs 100 mL
> Reservoir 400 mL
> NucleoMag SEP (Magnetic separator)

Required Consumables
> epT.I.P.S.® Motion 1000 µL with filter
> epT.I.P.S.® Motion 300 µL with filter

> NucleoMag RNA Kit
> Square-well Blocks
> Elution plate

Samples
2x 10^6 cells or up to 20 mg tissue

Method
This protocol is developed to process up to 96 samples in parallel on the ep*Motion* 5075m or 5075t automated liquid handling workstation.

The respective kit is suitable for up to 20 mg tissue or 2x 10^6 cells. The tissue samples can for example originate from mouse or other animals. Here the tissue samples were mouse liver, brain and heart. The tested cells were HeLa cells. Tissue and cells are lysed with Lysis buffer MR1 and TCEP. 350 µL lysed sample is prefilled into each well of the separation plate. All subsequent steps are automated and will be carried out in this plate. This includes dispensing of buffers and beads, removal of the supernatants as well as transport and mixing steps. After the lysis step magnetic beads and binding buffer MR2 are added. During a mixing and incubation step the RNA is bound to the magnetic beads. Beads are separated using the NucleoMag® SEP and the supernatant is removed. Unspecific bound contaminants are removed through three washing steps with wash buffers MR3 and MR4. After the last washing step residual ethanol is removed in a drying step of 10 minutes at 56° C on the Eppendorf ThermoMixer® of the ep*Motion*. Eventually the RNA is eluted and eluates are transferred to a dedicated elution plate.

The purification process with 96 RNA samples with re-use tip function for the wash steps requires 344x 1000µL tips and 104x 300 µL tips.

For the method the following positions of the worktable are occupied:

Position	Labware	Comment
A2	300 µL filtertips	
A3	300 µL filtertips	
TMX	Separation Plate (Lysed samples)	
B1	1000 µL filtertips	
B2	1000 µL filtertips	
B3	1000 µL filtertips	
B4	1000 µL filtertips	
C2	Liquid Waste (400 mL reservoir)	
C3	NucleoMag_SEP	
C4	ReservoirRack with reservoirs	
C5	Elution Plate	

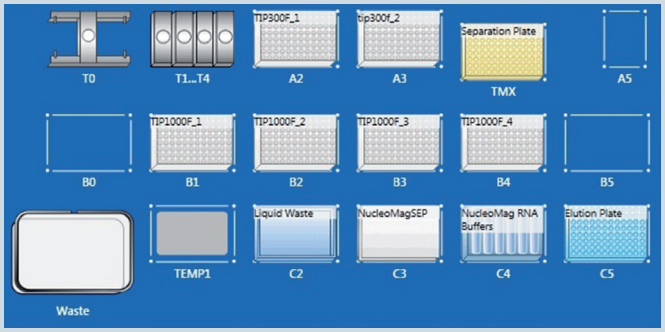

Figure 1: Worktable allocation

Figure 2: ReservoirRack Layout

Results and Discussion

Purification results from HeLa cells: RNA achieved with the aforementioned method was analyzed by UV spectroscopy demonstrating consistent and high yield with high purity (A260/A280 ratio >2) figure 3-4. Furthermore RT-qPCR analysis was used to check for the absence of inhibitors figure 5. In figure 6 and 7 results from UV spectroscopy and realtime PCR are summarized to verify the reliability of the application over broad range of starting cell material.

RNA from HeLa Cells

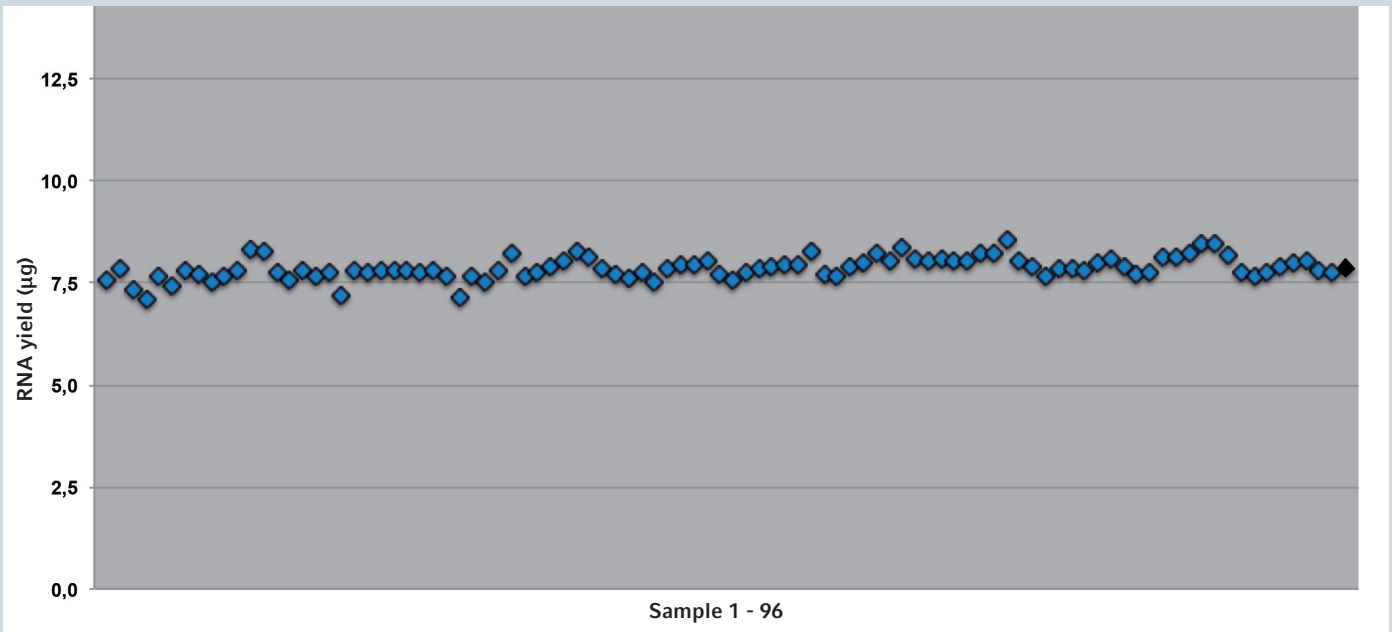

Figure 3: RNA yield (µg) from 96 HeLa cell samples ($5x10^5$ cells)

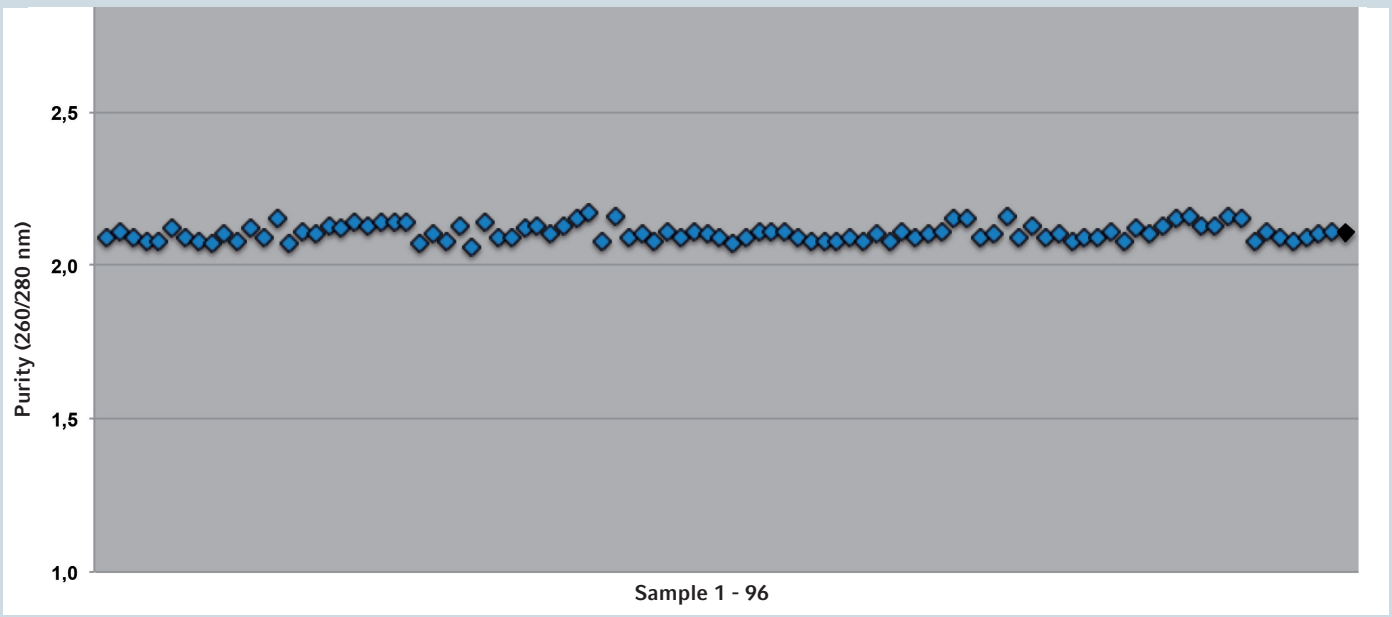

Figure 4: Purity shown as A260/A280 ratio of the extracted RNA from 96 HeLa cell samples.

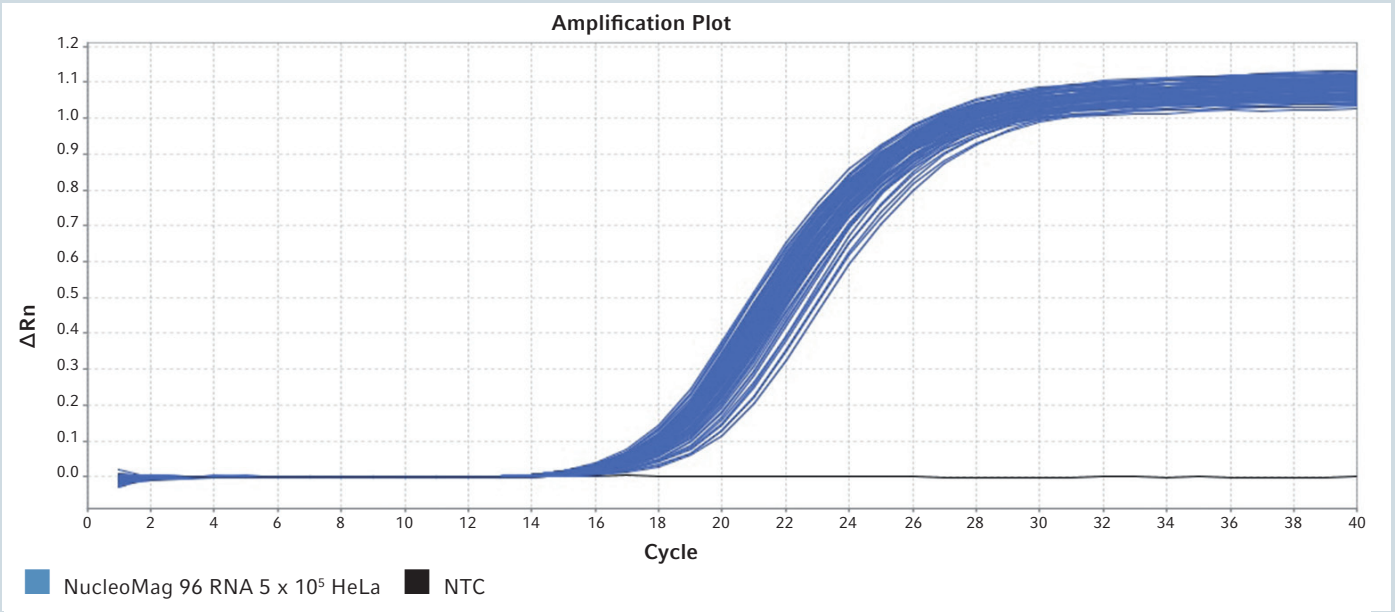

Figure 5: Amplification plot from 96 5 x10^5 HeLa cell samples. 4 µL of selected eluates were assayed in a quantitative RT-qPCR with a hydrolysis probe for a 130 bp beta-Actin amplicon (NTC = no template control)

Serial dilution of HeLa Cells

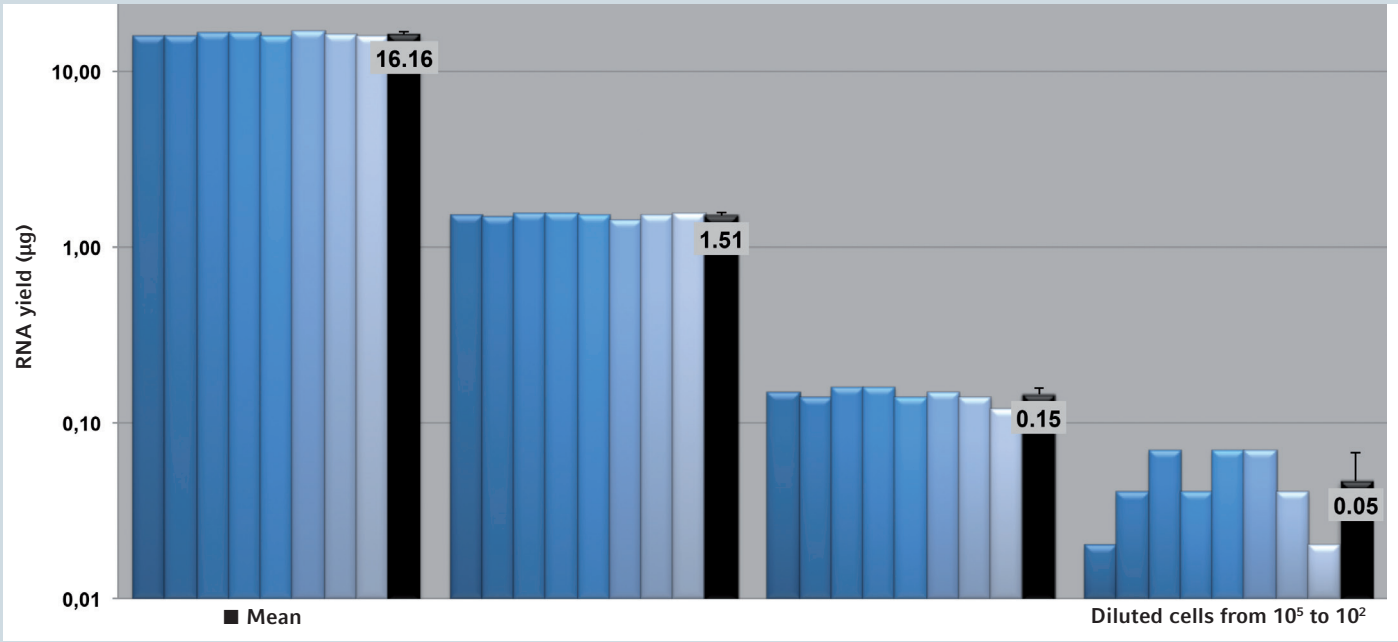

Figure 6: The proportional yield of a serial dilution of HeLa cells (106 to 103) shows the reliability of the application over broad amount range of starting material.

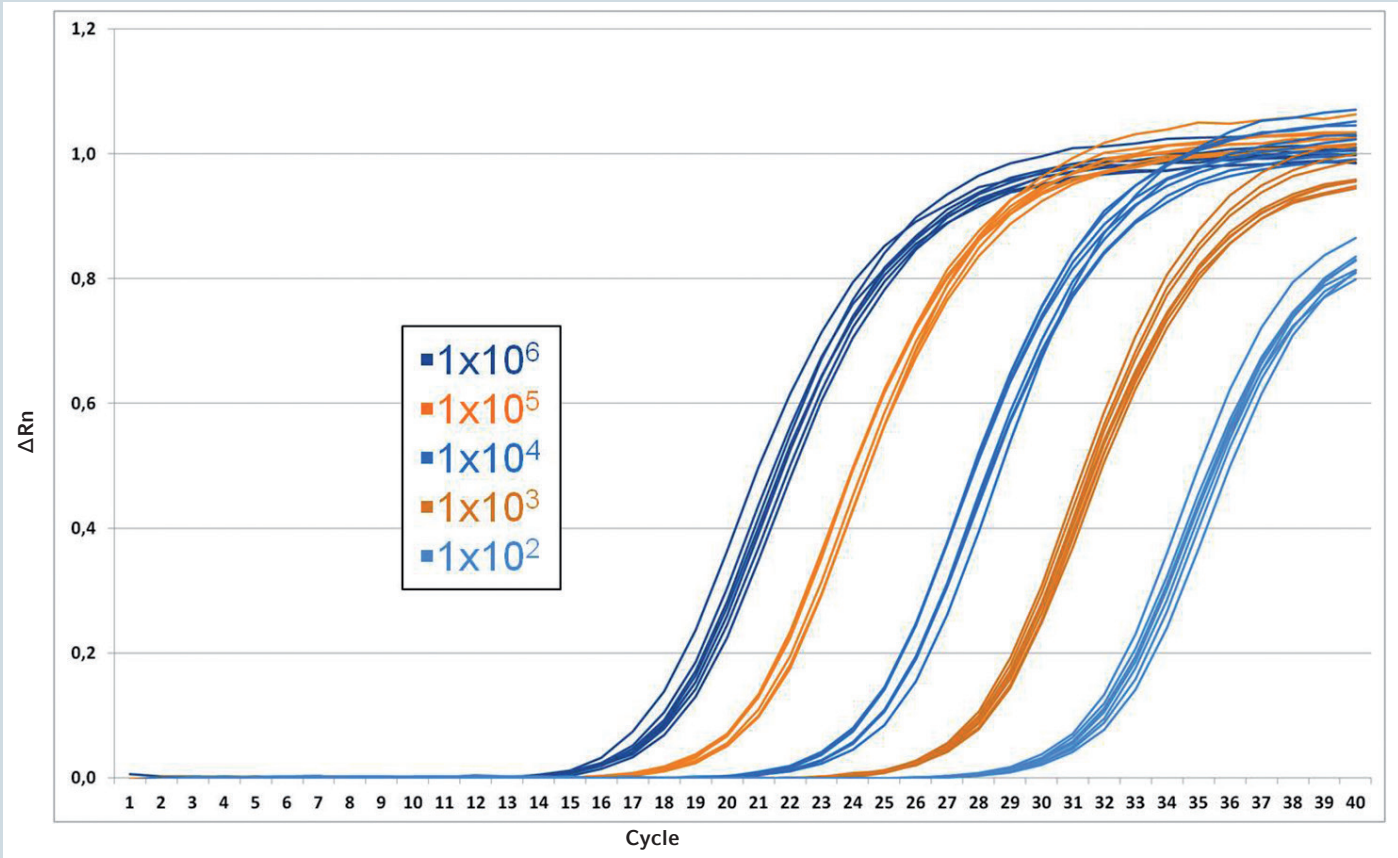

Figure 7: The diluted samples from HeLa cells. 4 µL of selected eluates were assayed in a quantitative RT-qPCR with a hydrolysis probe for a 130 bp beta-Actin amplicon.

Tissue

Purification results from mouse tissues (liver, brain, heart). RNA achieved with the aforementioned method was analyzed by UV spectroscopy. Furthermore a RT-qPCR was used to check for the absence of PCR inhibitors.

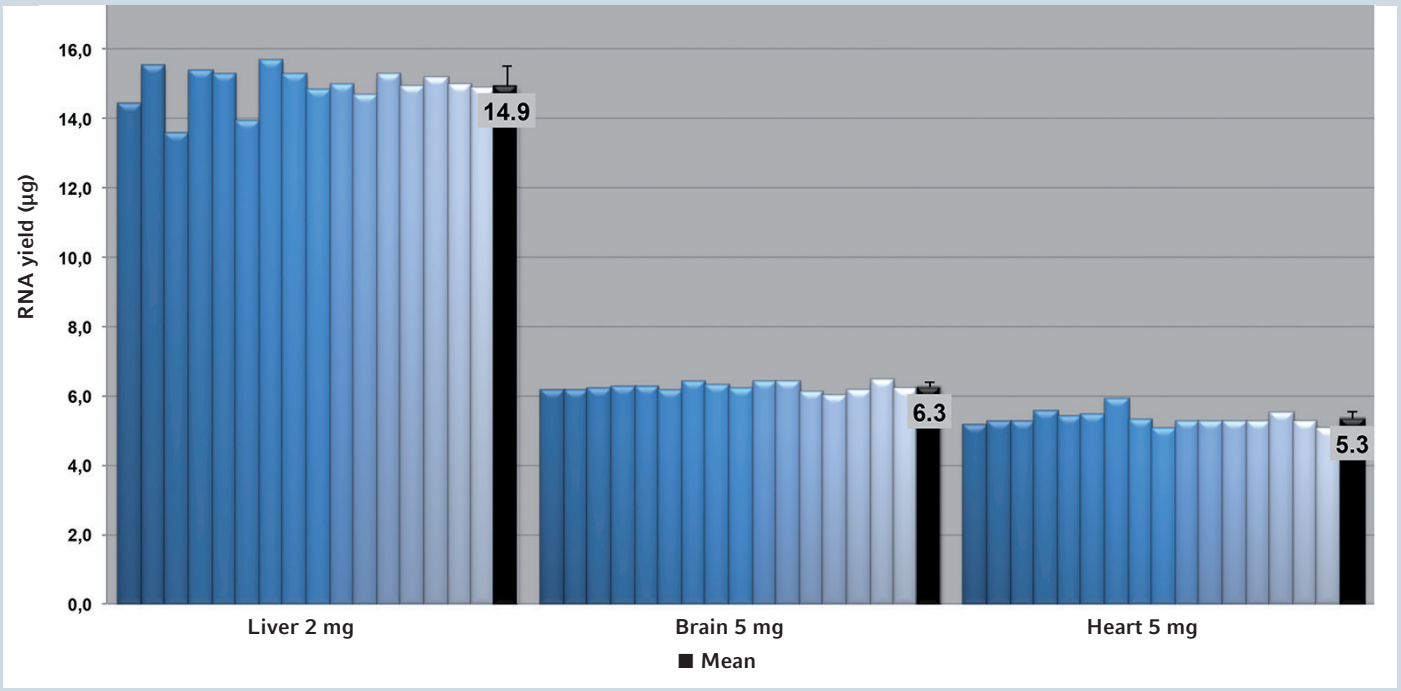

Figure 8: RNA yields from mouse tissue.

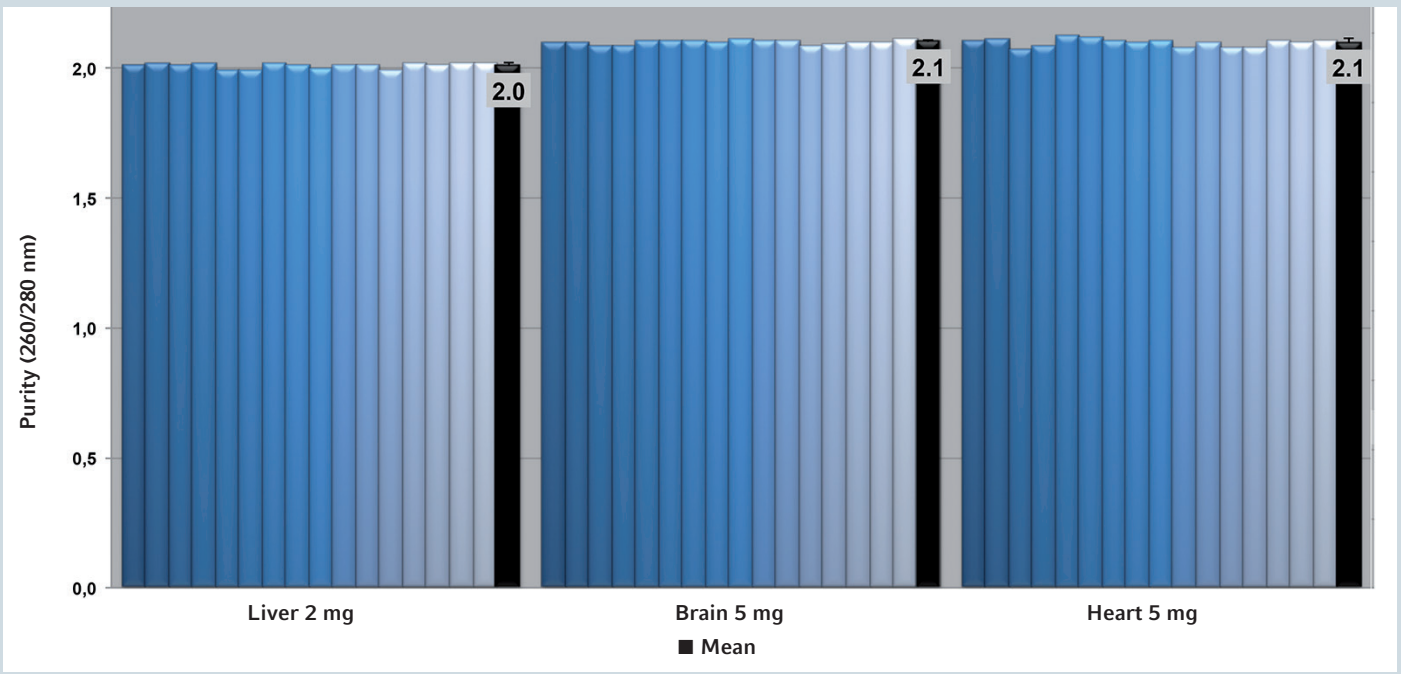

Figure 9: Purity (A260/A280) of RNA samples from mouse tissue.

Cross-contamination

HeLa cell samples were placed in the separation plate together with PBS buffer (negative control) in a checkerboard pattern.

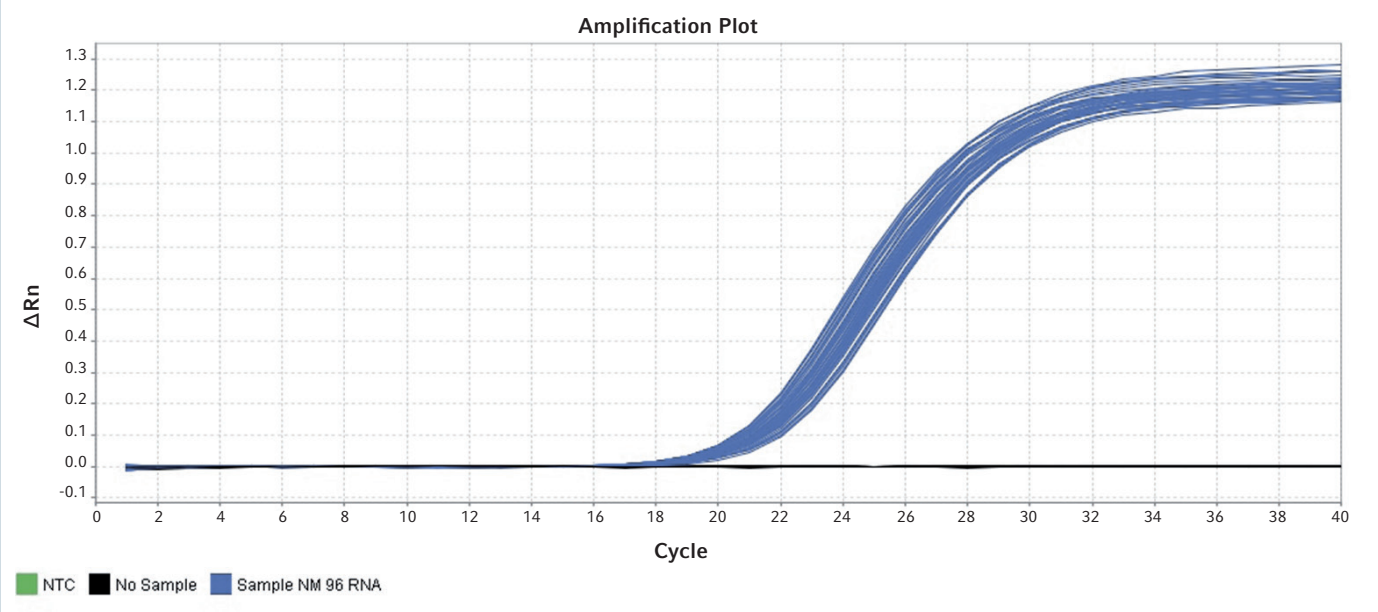

Figure 10: No PCR amplification could be observed in eluates from negative controls

Conclusion

The above results show that the combination of the NucleoMag RNA kit and the ep*Motion* 5075m/t reliably delivers high yields with high quality of RNA - from cells or tissue. No cross-contamination is detectable. The purified RNA is suitable for a full range of downstream methods. The results from the electrophoresis analysis, qPCR, purity and yield as well show the performance of the described procedure. The total time to process 96 samples is less than 100 minutes. The use of Eppendorf SafeRack along with the re-use function reduces the tip consumption, and thus needed deck space as well as cost per sample.

Ordering information

Description	Order no. international
epMotion® 5075t	5075 000.302
epMotion® 5075m	5075 000.305
ReservoirRack	5075 754.002
TM 1000-8 Dispensing tool	5280 000.258
TM 300-8 Dispensing tool	5280 000.231
Gripper with holder	5282 000.018
epT.I.P.S.® Motion 1000 µL SafeRack with filter	0030 014.650
epT.I.P.S.® Motion 300 µL with filter	0030 014.456
Reservoir 30 mL	0030 126.505
Reservoir 100 mL	0030 126.513
Reservoir 400 mL	5075 751.364

MACHEREY-NAGEL	
NucleoMag® RNA	REF 744350
NucleoMag® SEP	REF 744900
Square well block	REF 740481
Elution Plate U-bottom	REF 740486

Your local distributor: www.eppendorf.com/contact
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