High throughput RNA preparation using the QIAGEN RNeasy® 96 BioRobot® 8000 Kit on the workstation epMotion® 5075 from Eppendorf

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Introduction

With the wide application of RNA in expression profiling experiments, molecular diagnosis and RNAi, the need for high throughput RNA isolation has dramatically increased within the last few years. Eppendorf has addressed this demand with the integration of the well-established technology of the Qiagen RNeasy® 96 BioRobot® 8000 Kit into the workstation epMotion® 5075. This system allows the simultaneous isolation of 96 RNA samples from up to 5×10^5 animal or human cells per sample.

The RNeasy 96 BioRobot 8000 procedure replaces current time-consuming and tedious methods involving alcohol-precipitation steps, large numbers of washing steps, or the use of toxic substances such as phenol and/or chloroform. The purified RNA is ready to use in any downstream application including:

- RT-PCR
- Primer extension
- Northern dot blot analysis
- RNase/S1 nuclease protection
- Poly A+ RNA selection
- cDNA synthesis
- Quantitative Real-Time-PCR
- Differential display

Materials and methods

- Eppendorf epMotion 5075 Vac
- Vac Thermo Lid
- Reservoir 400 ml
- Collection Plate Adapter
- Channeling Plate
- Vac Frame 2
- Qiagen RNeasy 96 BioRobot 8000 Kit
- HeLa cells

Refer to the Qiagen RNeasy 96 BioRobot 8000 Kit manual for complete instructions on preparing working solutions.

Product use limitations and safety information

Please read the Qiagen RNeasy 96 BioRobot 8000 Kit manual before performing the method for the first time.

Culture and processing of the cells

5×10^7 HeLa cells were grown using standard cell culturing technique. The medium was removed and the adherent cells were resuspended in lysis buffer RLT. The suspension was pipetted up and down several times to lyse the cells. 160 µl of the cell lysis suspension corresponding to 5×10^5 cells were transferred to each well of an Eppendorf 96 deepwell plate for automated processing. The method RNeasy 96 of the epMotion software was used.

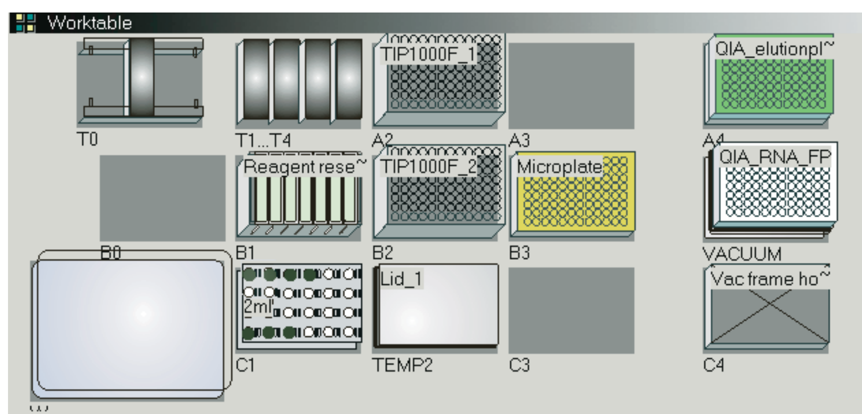

Figure 1: Screenshot from the epMotion Editor showing the setup of the epMotion 5075 Vac worktable for the protocol RNeasy 96.

Table 1: epMotion® 5075 VAC worktable details for the RNeasy 96 protocol

Position	Labware	Comment
A2	epT.I.P.S. Motion Filter 1000 µl	96 tips for 96 samples
A4	Qiagen Elution Plate	Collects eluate in 96 microtubes
	85 mm Adapter	Height adapter for Collection Plate
B1	Reagent Reservoirs	
	Position 1: Buffer RLT (optional)*	30 ml reservoir (optional)*
	Position 2: 70 % Ethanol	30 ml reservoir
	Position 3: Buffer RW1	100 ml reservoir
	Position 4: Buffer RPE	100 ml reservoir
	Position 5: Buffer RPE	100 ml reservoir
	Position 6: 96 % Ethanol	30 ml reservoir
	Position 7: RNase free water	30 ml reservoir
B2	ep T.I.P.S. Motion Filter 1000 µl	96 tips for 96 samples
B3	Microplate Deepwell	Contains cell samples
Vacuum	RNeasy filterplate	Filters lysate
	Vacuum Frame 2	Collar for vacuum chamber
	Reservoir 400 ml with channeling plate	Collects lysate and washes liquids
C1	24 Rack	
	Position A1-A4: DNase I Incubation mix	2 ml Eppendorf tubes
	Position D1-D4: Top Elute Fluid	2 ml Eppendorf tubes
C2	Vac Thermo Lid	Heated lid for vacuum
C4	Vacuum Frame Holder	Height adapter for Vacuum Frame 2
T1	TM 1000-8	1000 µl 8-channel pipetting tool
T2	TS 10000	1000 µl single channel pipetting tool

*Lysis of the cells is done before starting the automated protocol, but may also be integrated into the procedure. Please contact the Eppendorf application hotline for further assistance.

Table 2: Reaction conditions for reverse transcription of two-step RT PCR

Components	Volume	Final concentration in the reaction
Mastermix 1	**10 µl**	
RNase-free H_2O	1 µl	
dNTP mix [10 mM each]	2 µl	1 mM
Random hexamers (50 ng/µl)	1 µl	2.5 ng/µl
Template RNA	3 µl	150 ng – 1 µg total RNA
Mastermix 2	**10 µl**	
RNase-free H_2O	4.5 µl	
RTplus PCR Buffer with 25 mMg^{2+}	4 µl	2x with 5 mM Mg^{2+}
cMaster RT Enzyme	1 µl	0.75 U/µl
Prime RNase Inhibitor Solution	0.5 µl	0.025 U/µl

Table 3: Reaction conditions for the second-step PCR reaction

Components	Volume	Final concentration in the reaction
Mastermix 3	**10 µl**	
RNase-free H_2O	5 µl	
dNTP mix [10 mM each]	1 µl	200 µM
Forward primer	1 µl	0.4 µM
Reverse primer	1 µl	0.4 µM
First step RT reaction mix	2 µl	N/A
Mastermix 4	**40 µl**	
RNase-free H_2O		
RTplus PCR Buffer with 25 mM Mg^{2+}	5 µl	1x with 2.5 mM Mg^{2+}
cMaster PCR Enzyme Mix	0.4 µl (2 U)	0.04 U/µl

Table 4: Incubation program for first strand cDNA synthesis

Step	Temp.	Duration
Template RNA denaturation	65°C	5 min
Fixation of the RNA	0°C	5 min
Addition of Mastermix 2 (10 µl)		
Hexamer primer annealing and extension	25°C	10 min
First strand cDNA synthesis	42°C	50 min
cDNA synthesis	50°C	50 min

Reverse transcription PCR analysis

RNA samples from the 96-well plate were analyzed with RT-PCR using the Eppendorf cMaster RT_{plus} PCR system. The reaction conditions and the cycling parameters are listed in tables 2 to 5. The protocol described in the cMaster RT_{plus} PCR system manual was followed. Please refer to the manual for complete instructions on the procedure. Specific primers for the amplification of a 372 bp product of the human ß-globin mRNA were used.

Table 5: Incubation program for second step PCR

Step	Temp.	Duration	Cycles
Initial template denaturation	94°C	2 min	1 x
Denaturation	94°C	20 sec	35 x
Primer annealing	62°C	20 sec	
Elongation	68°C	20 sec	
Final elongation	68°C	2 min	1 x

Results

Formaldehyde agarose gel analysis

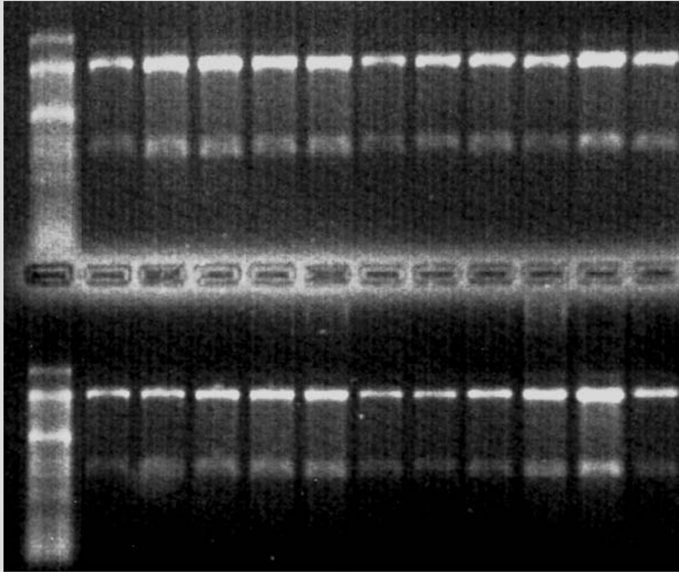

Figure 2: Total RNA was isolated from approx. 5x10^{5} HeLa cells 10 µl each of 22 samples were run on a 1% formaldehyde agarose gel and stained with ethidium bromide. The size marker is RNA marker I (Roche Applied Science).

Yield and purity

Depending on the cell line and growth conditions applied, the actual yield obtained with the RNeasy procedure can vary. The RNA amounts for the recommended numbers of cells are significantly below the maximum binding capacity of each well. The average concentration obtained with approx. 5 x 10^{5} HeLa cells was 215 ng/µl per well with an average $A_{260/280}$ ratio of 1.92.

Table 6: Average yield and purity of total RNA

Number of cells	5 x 10^{5} HeLa cells
$A_{260/280}$	1.92
Concentration (ng/µl)	215.2
Theoretical Yield (µg)	12.91

Reverse transcription PCR analysis

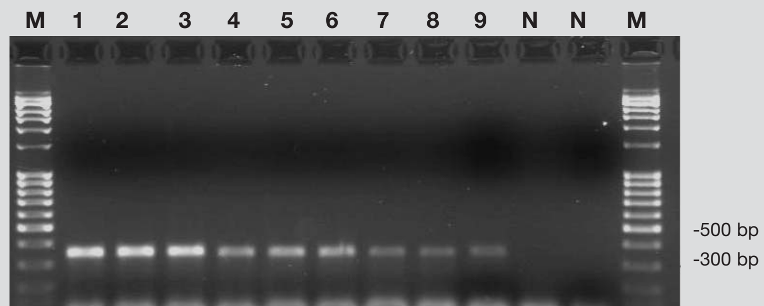

Figure 3: RT-PCR analysis of decreasing amounts of total RNA of various samples (lanes 1-9) derived from one 96 plate. The size marker is 100 bp DNA-ladder, extended (Roth). Isolated RNA was subjected to reverse transcription as described. 1/10 of this reaction was used for second step PCR analysis. As a negative control, PCR was performed with the respective RNA preparations in the absence of reverse transcription (lanes N). A 372 bp fragment of human beta-actin was amplified using intron spanning primers (contamination with genomic DNA would lead to the amplification of a 500 bp fragment).

Conclusion

The Qiagen RNeasy® 96 BioRobot® 8000 Kit integrated into the workstation epMotion® 5075 offers a complete system for automated purification of high quality total RNA from 96 samples. The procedure is very easy to perform, user-friendly and yields RNA suitable for various downstream applications. With HeLa cells, approximately 12 µg of total RNA with an $A_{260/280}$ ratio of 1.92 could be isolated from 5 x 10^{5} cells. The epMotion 5075 can easily process one 96-well plate in approximately 90 minutes. No genomic DNA contamination could be detected, indicating the quality of the isolated RNA. With the RNeasy technology, the broad application spectrum of the epMotion® 5075 VAC workstation is extended to high throughput RNA isolation.

References

Eppendorf
Instrument Manual for the epMotion® 5075 Vac
Guidelines for processing the Qiagen RNeasy® 96 BioRobot® 8000 Kit on the epMotion® 5075 Vac workstation.

Qiagen
RNeasy® 96 BioRobot® 8000 Handbook

Ordering information Eppendorf

Product	International order no.	North American order no.
Vac Thermo Lid	5075 796.007	960002551
Reservoir 400 ml	5075 777.002	960002229
Collection Plate Adapter	5075 785.030	960002531
Channeling Plate	5075 794.004	960002540
Vac Frame 2	5075 785.005	960002261
epMotion® 5075 Vac 230 V (vacuum chamber included)	5075 000.164	n/a
epMotion® 5075 Vac 120 V (vacuum chamber included)	5075 000.164	960020014

Ordering information Qiagen

Product	order no.
Qiagen RNeasy® 96 BioRobot® 8000 Kit	967152

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