MagNA Pure 96 Cellular RNA Kits: RNA Isolation on the Fast Lane

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Introduction

Advancements in high-throughput gene expression analysis have set a new demand for automated isolation of cellular RNA from high sample numbers in parallel. The availability of the innovative MagNA Pure 96 System together with dedicated kits, protocols, and consumables sets a new standard in truly walkaway high-throughput automated nucleic acid sample preparation.

Here we describe the performance of the MagNA Pure 96 Cellular RNA Small and Large Volume Kits for total RNA isolation from cultured cell samples. As with all members of the MagNA Pure System family, nucleic acid is isolated by efficient lysis of the biological material and binding of nucleic acids to magnetic glass particles (MGP) in the presence of a strong chaotropic salt. The bound nucleic acid is washed several times prior to elution in a low-salt aqueous elution buffer. Table 1 displays an overview of the basic application parameters. All kit components but one are formulated and packed in a ready-to-use format. The lyophilized DNase, used for digestion of the DNA during sample processing, needs to be reconstituted with a supplied DNase incubation buffer. The loading of the two reagent trays, the two bottles of MGP, and the DNase bottle, as well as the required disposable plates and tips, is fast and straightforward. Other system characteristics have been described in an earlier article in Biochemica (4/2009, page 29).

Materials and Methods

Cellular RNA was isolated from serial dilutions of cultured K562 and HeLa cells. Cells were stored as frozen cell pellets with 10^7 cells/vial at -80°C. Cell pellets were mixed gently on ice with cold PBS buffer by pipetting up and down. The indicated number of cells/sample were prepared and the sample was transferred to the MagNA Pure 96 Processing Plate. Either the *RNA Cells SV* or the *RNA Cells LV* protocol was selected and the desired elution volume was set. The RNA isolation for 96 samples was completed in 62 (small volume) or 80 (large volume) minutes.

To analyze yield and quality of the isolated RNA the following methods were used:

- Agarose gel electrophoresis using 1% agarose MP in 1x Tris/borate buffer, 12 µl SYBR® Green II RNA stain/100 ml agarose gel. The gel was run for 1 hour at 120 volts.
- Optical density (OD) measurements at 260 nm and 280 nm using the NanoDrop 8000 Instrument
- 2100 Bioanalyzer RNA Nano 6000 kit.

Real-time RT-PCR and PCR were performed using the LightCycler® 480 System and the LightCycler® RNA Amplification Kit HybProbe or the LightCycler® RNA Amplification Kit Hydrolysis Probe.

Results and Discussion

Table 2 summarizes the yields achieved when the MagNA Pure 96 Cellular RNA Small and Large Volume Kits for

Table 1: Basic applications parameter

Kit name	Number of cultured cells per isolation	Possible elution volume
MagNA Pure 96 Cellular RNA Small Volume Kit (6x96 reactions)	≤ 5×10^4 cultured cells in 100 µl PBS	50 µl, 100 µl
MagNA Pure 96 Cellular RNA Large Volume Kit (3x96 reactions)	≤ 1×10^6 cultured cells in 200 µl PBS	50 µl, 100 µl, 200 µl

Table 2: Yield from HeLa and K562 cell samples. RNA was isolated either using the MagNA Pure Small or Large Volume Kit as indicated.

	HeLa		K562		
Number of cells	Yield [ng] RT-PCR	Yield [ng] OD	Yield [ng] RT-PCR	Yield [ng] OD	Kit type
1.00E+06	1712	2880	8422	17800	Large volume
1.00E+05	232	200	922	1900	Large volume
5.00E+04	224	243	586	712	Small volume
1.00E+04	30.2 53.2		109 121		Large volume Small volume
1.00E+03	4.8		16.2		Small volume
5.00E+02	2.2		6.2		Small volume

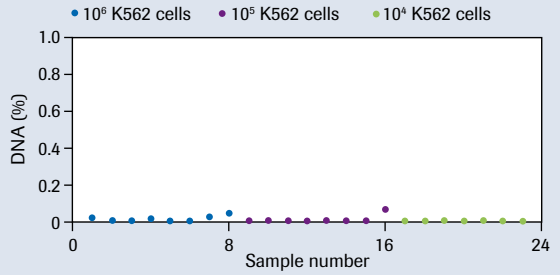

Figure 1: Residual DNA. Amount of DNA was determined by CycA qPCR assay and calculated as proportion of total nucleic acid content measured by optical density.

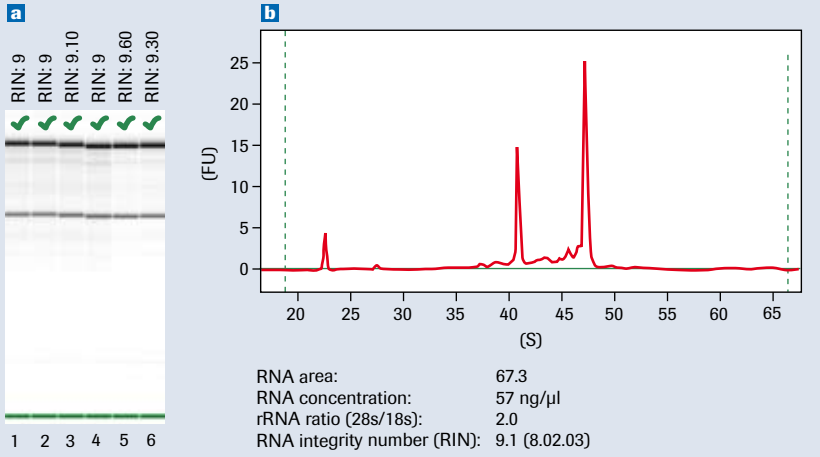

Figure 2: Characterization of isolated RNA using a commercially available platform. (a) RNA from 1.00E+06 HeLa cells was isolated using either the MagNA Pure 96 (samples 1-4) or the MagNA Pure LC Instrument (samples 5, 6) and run on the RNA Nano 6000 Kit. **(b)** Chromatogram of an exemplary RNA sample**.**

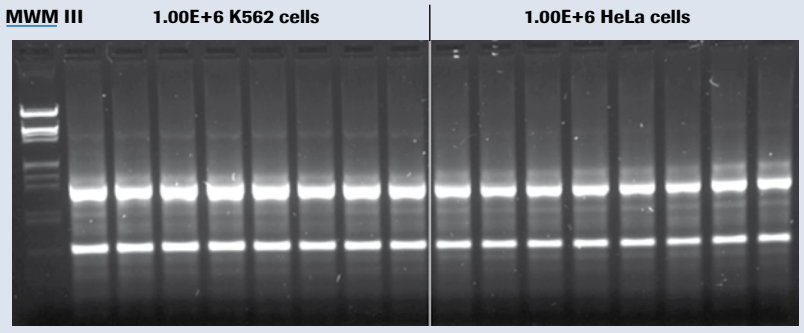

Figure 3: Characterization of isolated RNA by agarose gel electrophoresis. Isolated RNA from 1.00E+06 HeLa or K562 samples was diluted 1:10 (K562) or 1:2 (HeLa) and run through a 1% agarose gel in 1 x TBE buffer. The agarose gel contained 12 µl/100 ml of the RNA stain SYBR® Green II.

isolating RNA from two sample types with different cell counts were used. Note the good scalability and the similarity for the 1.00E+04 cell count for both kit types. The $OD_{260/280}$ ratio for the samples measured with the NanoDrop Instrument was between 2.0 and 2.2.

The isolated RNA is of high purity and has no or only a very low level of residual DNA. The bulk part of genomic DNA is removed by an integrated DNase digestion step within the system protocol. We tested for the amount of residual DNA using a very sensitive PCR assay that included the CycA gene and pseudogenes as targets. Typically, no DNA was detectable during 45 cycles on the LightCycler® 480 System. In cases in which DNA was detected, the amount was well below 0.1% of the amount of total nucleic acids (Figure 1).

RNA quality can also be assessed by looking at the integrity of the isolated RNA. We analyzed our samples with a commercially available platform that uses capillary electrophoresis on chips. The RNA integrity number (RIN) is automatically calculated by the system software using a proprietary algorithm. An RIN between 7 and 10 characterizes an RNA sample as suitable even for highly demanding array experiments. Using the MagNA Pure 96 System, RNA with an RIN of ~9.0 can be obtained; this is comparable to a typical result achieved when using the MagNA Pure LC RNA Isolation Kit, High Performance (Figure 2). Electrophoresis of isolated RNA through a 1% agarose gel gives a more qualitative picture: clear and distinct intact ribosomal 18S and 28S bands are detected with RNA isolated from HeLa and K562 cell samples (Figure 3).

Finally, these RNA samples should perform well in real-time qRT-PCR analysis. We routinely analyzed the RNA eluates using three different assays on the LightCycler® 480 Instrument and a one-step RT-PCR master mix. The CycA assay uses the Hybridization Probe format; the mRNA coding for β2M and GAPDH was amplified and detected by a hydrolysis probe from the Universal Probe Library (UPL) product line (UPL number 32 and 60, respectively). We obtained very uniform and clear sigmoid amplification curves on the LightCycler® 480 System (Figure 4). In addition, dilution of initial cell pellets showed a good linearity of respective crossing points (Cp).

To check for potential RT-PCR inhibitors, we performed dilution experiments of the RNA eluates. Tenfold dilution of RNA samples resulted in the expected Cp shift between 2.8 and 3.5 (should be around 3.3). The MagNA Pure 96 protocols for cellular RNA isolation *RNA Cells SV* and *RNA Cells LV* allow specified settings to elution volumes of 50 µl, 100 µl, and 200 µl (LV only). When a smaller elution volume is used, the RNA concentration is increased; in some instances, however, the total amount of isolated RNA might drop. Typically,

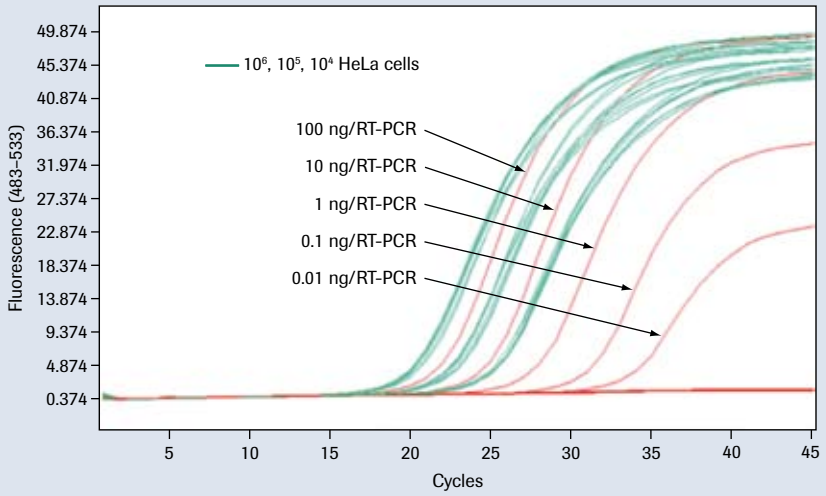

Figure 4: qRT-PCR analysis. Cellular RNA from a serial dilution of HeLa cells was isolated and 5 µl of the eluate was amplified in real-time qRT-PCR for β2M mRNA using the LightCycler® 480 System. A standard curve was generated using a dilution series of a HeLa RNA standard.

at least 80% of the total amount of RNA is obtained in 50 µl as compared with 100 µl elution volume, thus increasing the concentration by ~60%.

Conclusion

The MagNA Pure 96 System allows – for the first time – fast and efficient cellular RNA isolation in a high-throughput format. The isolated RNA is of high quality and purity, allowing highly demanding array applications as well as routine real-time qRT-PCR analysis. The kit is available as a convenient ready-to-use format. The automation reduces human error and variation in the isolation process, thus increasing the reproducibility and the standardization of the results. Future applications include the isolation of cellular RNA from whole blood (external lysis) and whole blood samples stabilized in PAXgene® tubes. Please find additional related information on the system home page at www.magnapure96.com. ■

Order INFO

Product	Pack Size	Cat. No.
LightCycler® RNA Amplification Kit HybProbe	1 kit (96 reactions of 20 µl final volume)	12 015 145 001
LightCycler® RNA Master Hydrolysis Probes	1 kit (5 x 100 reactions of 20 µl final volume)	04 991 885 001
MagNA Pure 96 Cellular RNA Small Volume Kit	3 x 192 purifications	05 467 543 001
MagNA Pure 96 Cellular RNA Large Volume Kit	3 x 96 purifications	05 467 535 001