fields and closing the door cover, the run is started. Once the run is finished, the Result screen displays the run documentation, which can be saved, printed out, or routed via LAN. ■

Order INFO

Product	Pack Size	Cat. No.
MagNA Pure Compact Instrument includes internal PC with touch screen and bar-code scanner	1 instrument	03 731 146 001
MagNA Pure Compact Nucleic Acid Isolation Kit I for 32 isolations of genomic DNA from mammalian whole blood or culture cells, and viral nucleic acids from plasma or serum in the range of 100 µl – 400 µl (including all plastic disposables needed)	1 kit	03 730 964 001
MagNA Pure Compact Nucleic Acid Isolation Kit I – Large Volume for 32 isolations of genomic DNA from mammalian whole blood or culture cells, and viral nucleic acids from plasma or serum in the range of 500 µl – 1,000 µl (including all plastic disposables needed)	1 kit	03 730 972 001
MagNA Pure Compact Tip Tray Kit for the Leakage Test and as a replacement	1 kit (10 tip trays)	03 753 166 001
MagNA Pure Compact RNA Isolation Kit		Coming soon!

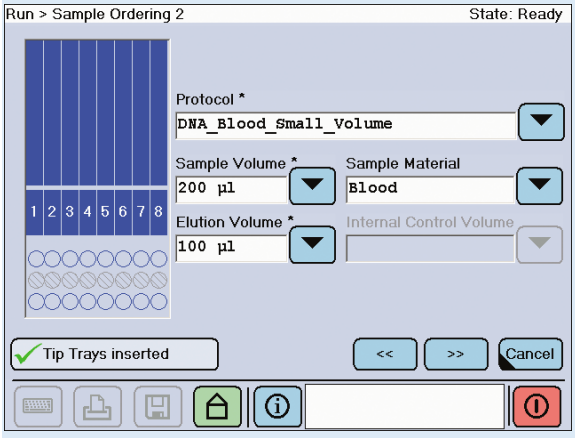

Figure 2: Sample Ordering 2 Screen – Selection of the protocol and its parameters. Convenient pull-down menus show the protocols and parameters suitable for the appropriate isolation kit.

The New MagNA Pure Compact Nucleic Acid Isolation Kits – Fast and Flexible Fully Automated Sample Preparation

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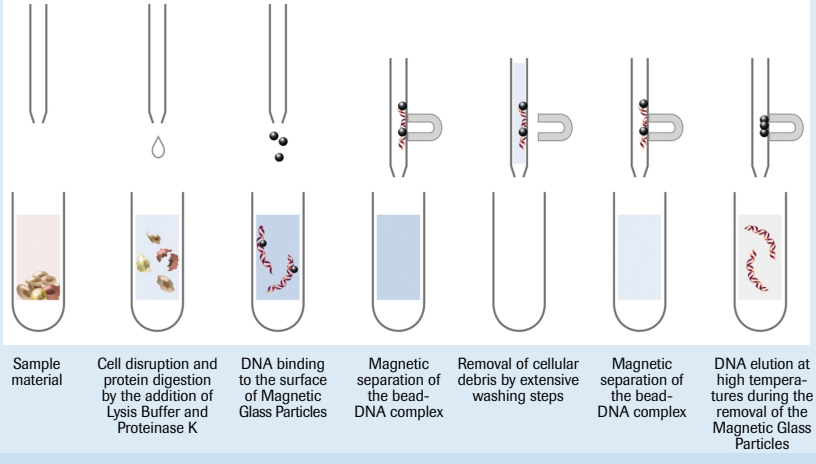

Figure 1: Principle of nucleic acid isolation performed automatically by the MagNA Pure Compact Instrument

Introduction

Two kits for the newly developed MagNA Pure Compact System, together with a large variety of protocols, provide a previously unknown flexibility regarding sample type, sample and elution volume, and target nucleic acid (Table 1). Furthermore, an internal control that is automatically integrated into the process can be used. The ready-to-use reagent kits contain prefilled reagent cartridges and all the disposables necessary.

All nucleic acid isolation steps are performed automatically by the MagNA Pure Compact Instrument, based on the proven magnetic-bead technology as established with the MagNA Pure LC Instrument (Figure 1).

The reagents and protocol steps are optimized to achieve a high nucleic acid yield from mammalian blood, plasma, serum or culture cells. The whole isolation procedure takes only 22 – 35 minutes depending on the protocol.

The new kits were tested with human blood from different donors, plasma spiked with DNA and RNA viruses, and different culture cell lines. The tests included criteria such as DNA integrity, yield, purity, reproducibility, scalability, cross contamination and comparison to other methods.

Materials and Methods

Samples

Blood research samples (EDTA blood) from different human donors as well as blood pools were used. Plasma (citrate) was spiked with a dilution series of parvovirus B19 and hepatitis A virus (HAV) prior to nucleic acid isolation. Culture cells (K562, HeLa) were pelleted and then resuspended in phosphate-buffered saline (PBS).

DNA isolation on the MagNA Pure Compact

Reagent Cartridges from the respective MagNA Pure Compact Kit were scanned by the bar-code scanner and loaded into the Intrument Cartridge Rack. Tip Trays and bar-coded Elution Tubes from the kit were placed in the respective positions. Then whole blood or plasma (100 µl – 1 ml) or culture cell suspensions were directly transferred to the sample tubes and inserted in the Tube Rack. The respective protocol, sample and elution volumes were chosen from the software, and the automated nucleic acid isolation was started. The MagNA Pure Compact Instrument automatically performs all isolation steps such as cell lysis, protein digest, binding of DNA, washing steps, and elution of the pure nucleic acid (Figure 1).

Analysis of the isolated DNA

The integrity of the isolated DNA was checked on agarose gels together with DNA Molecular Weight Marker III (Roche Applied Science). The DNA yields were calculated by optical density (OD) measurement at 260 nm, the purity was assessed by calculating the ratio $OD_{260\,nm}/OD_{280nm}$. Furthermore, LightCycler PCRs or RT-PCRs (for HAV) were performed using the LightCycler research kits for HER2/*neu* DNA quantification, parvovirus B19 quantification, or HAV quantification (all Roche Molecular Diagnostics).

Results and Discussion

DNA integrity

Agarose gel analysis of the DNA isolated from blood showed a high integrity (Figure 2). The molecular weight was > 20 kb.

Table 1: a) MagNA Pure Compact Nucleic Acid Isolation Kit I

Sample type	Sample volume	Target nucleic acid	Optional internal control	Elution volume
whole blood	100 µl, 200 µl, 300 µl, 400 µl	genomic DNA, viral nucleic acid	yes	100 µl or 200 µl
plasma/serum	100 µl, 200 µl, 300 µl, 400 µl	total NA, viral nucleic acid	yes	50 µl or 100 µl
culture cells	100 µl with ≤ 5×10^5 cells	genomic DNA, viral nucleic acid	no	200 µl

b) MagNA Pure Compact Nucleic Acid Isolation Kit I – Large Volume

Sample type	Sample volume	Target nucleic acid	Optional internal control	Elution volume
whole blood	500 µl, 1000 µl	genomic DNA, viral nucleic acid	yes	100 µl or 200 µl
plasma/serum	500 µl, 1000 µl	total NA, viral nucleic acid	yes	50 µl or 100 µl
culture cells	100 µl with ≤ 10^6 cells	genomic DNA, viral nucleic acid	no	200 µl

Table 2: DNA yields from different sample materials and volumes. Note that the DNA yield from blood strongly depends on the blood donor and the blood cell count. The yield from culture cells depends on the cell line due to the variable degree of aneuploidy.

Sample material	Volume or amount	DNA yield
Blood (5,600 leucocytes/µl)	100 µl	4.6 µg
Blood (5,600 leucocytes/µl)	200 µl	7.5 µg
Blood (5,600 leucocytes/µl)	300 µl	10.3 µg
Blood (5,600 leucocytes/µl)	400 µl	12.0 µg
Blood (5,600 leucocytes/µl)	500 µl	14.1 µg
Blood (5,600 leucocytes/µl)	1,000 µl	30.1 µg
K562 cells	10^6 in PBS	32.0 µg
HeLa cells	10^6 in PBS	30.0 µg

Yield and purity

OD analysis revealed yields of up to 30 µg genomic DNA from 1 ml blood, and up to 32 µg from 10^6 culture cells (Table 2). Yields were similar to or higher than the values obtained by other purification methods, e.g., filter tubes. The $OD_{260\,nm}/OD_{280nm}$ ratio was ≥1.8, indicating a DNA

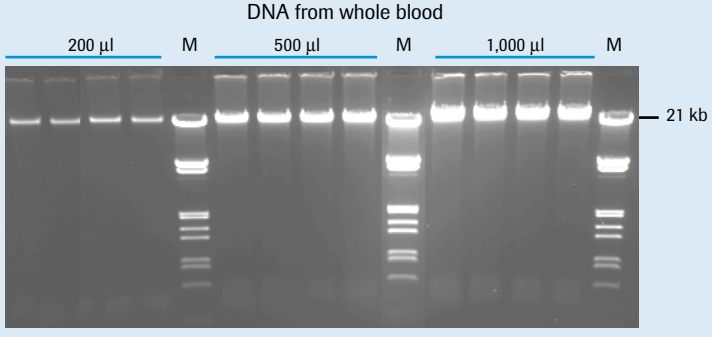

Figure 2: DNA Integrity. DNA was isolated from 200 µl, 500 µl and 1,000 µl of blood in fourfold replicates, respectively, and analyzed on a 0.8 % agarose gel. Comparison with DNA Molecular Weight Marker III (M) revealed a molecular weight of >20 kb.

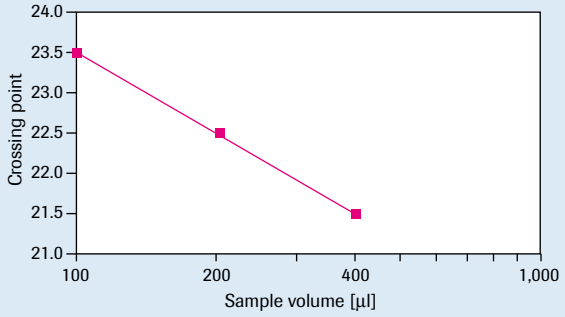

Figure 3: LightCycler quantitative PCR. The isolated DNA was amplified by real-time PCR in the LightCycler Instrument using the LightCycler HER2/*neu* DNA Quantification Kit intended for life science research as an example. The resulting LightCycler crossing points confirmed the excellent scalability of the isolation procedure. Due to the nature of the PCR amplification (2^n) there should be a linearity of crossing points regarding the logarithm of template amount. Each doubling of the template should reduce the crossing point by 1. This linearity was met perfectly.

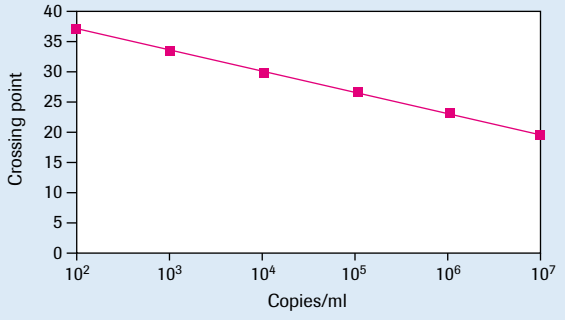

Figure 4: Sensitivity. Plasma spiked with parvovirus B19 in different concentrations was processed and eluates were analyzed in a parvovirus-specific LightCycler PCR. The resulting crossing points revealed an excellent sensitivity and linearity.

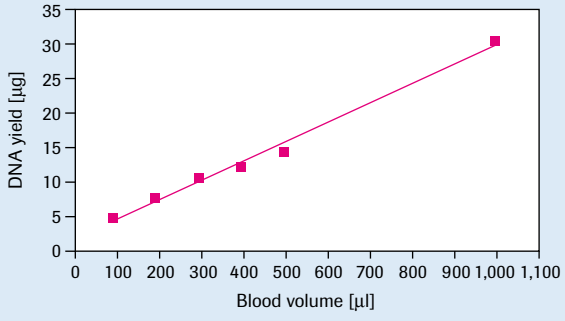

Figure 5: Scalability of yield from different sample volumes. DNA was isolated from 100 µl – 1,000 µl of whole blood and eluted in 200 µl. Yields were proportional to the sample input volume.

of high purity. No inhibition was detected in the LightCycler PCR (Figures 3 and 4).

Scalability

The DNA yields from different sample amounts (e.g., 100 µl – 1,000 µl blood) showed an excellent scalability (Figure 5).

Sensitivity

Citrate plasma was spiked with a dilution series of the human parvovirus B19 (DNA virus) and HAV (RNA virus) in the range of 10^2 – 10^7 copies per ml of plasma. For nucleic acid isolation, 200 µl of each spiked plasma was used. 5 µl of the total elution volume of 100 µl were used in the respective LightCycler PCR or RT-PCR (theoretically corresponding to 1 – 10^6 copies per [RT-]PCR, assuming a 100 % recovery). Figure 4 shows the results with parvovirus B19. Down to 100 copies/ml corresponding to 1 copy per PCR could be detected, showing an excellent sensitivity and linearity.

Variable elution volume

A variable elution volume is possible for blood (choice of 100 µl or 200 µl) and plasma/serum (choice of 50 µl or 100 µl). For culture cells, this option is not included at this point due to the very high DNA concentration.

Conclusion

The new MagNA Pure Compact Nucleic Acid Isolation Kits have proven to be useful and versatile tools for efficient, automated isolation of nucleic acids from various sample types and various amounts of material. All isolation steps are performed automatically by the MagNA Pure Compact Instrument within 22 – 35 minutes, depending on the protocol that was chosen.

The isolated nucleic acids were of high quality and showed no PCR inhibition. Typical yields from 1 ml blood were 17 – 30 µg, depending on the donor. The yields from 10^6 culture cells were up to 32 µg, depending on the cell line. The reproducibility was excellent and no cross contamination was detected. Thus the new MagNA Pure Compact Nucleic Acid Isolation Kits are valuable and reliable tools for nucleic acid isolation and analysis. ■