



MagNA Pure LC DNA Isolation Kit – Large Volume: A New Flexible Tool for Automated Sample Preparation

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Introduction

Many kits and protocols for nucleic acid isolation allow the processing of sample volumes in the range of 200 µl. This is also true for the majority of automated sample preparation procedures. However, many research applications require higher starting volumes and higher DNA yields. Therefore, the new MagNA Pure LC DNA Isolation Kit – Large Volume was developed for the processing of up to 1 ml of whole blood per isolation, and for large amounts of culture cells. This kit provides a high degree of flexibility, as it can be used together with four different MagNA Pure LC protocols.

All steps of DNA isolation are automatically performed by the MagNA Pure LC Instrument. The reagents and protocol steps were optimized for processing large amounts of blood and culture cells, while achieving maximum DNA yield and purity. The kit was tested with blood from different donors, including lipemic blood, and with different culture cell lines. 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 from eight different donors and blood pools were used. Two of the single blood samples were lipemic and therefore more difficult to process. The protocol for culture cells was tested with aliquots of 10^6 – 5×10^6 cells of various cell lines.

DNA isolation on the MagNA Pure LC Instrument

Blood (various volumes, 20 µl–1 ml) was directly transferred to the sample cartridge of the MagNA Pure LC Instrument. Prior to use, culture cell pellets were resuspended in 100 µl phosphate-buffered saline (PBS). The respective protocol was chosen from the software, the necessary plastic disposables and kit reagents were loaded onto the workstation, and the automated DNA

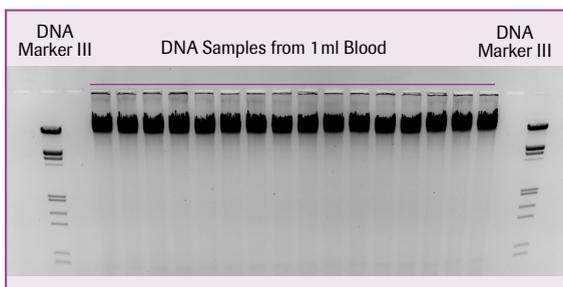


Figure 1: Agarose gel analysis of 16 DNA research samples isolated from 1 ml blood; 10 µl eluate were used per lane.

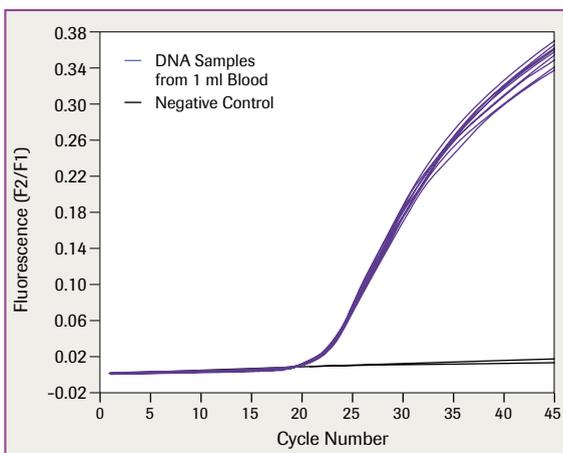


Figure 2: Reproducibility. LightCycler PCR analysis of 15 DNA research samples isolated from 1 ml blood. 5 µl eluate were used per PCR. The curves show a very low variation of Crossing Points.

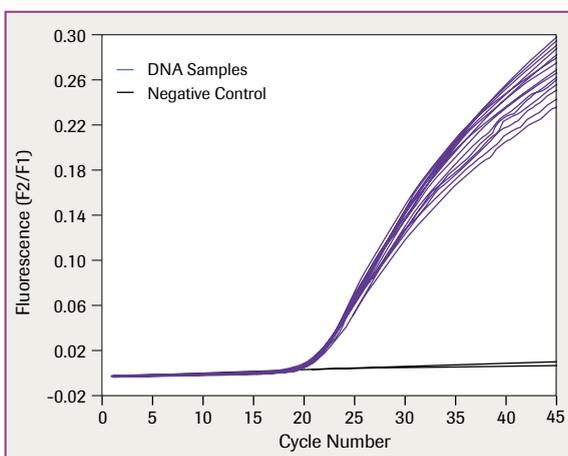
Table 1: Yield, purity, and LightCycler PCR results of DNA isolated from different blood donors, from a blood pool and from four culture cell lines. All results are mean values of 4–32 replicates.

Blood donor/type or culture cell line	Sample input	DNA yield (µg)	Purity (OD _{260nm/280nm})
blood pool 1 (10 donors)	1 ml	25.0	1.8
blood pool 2 (10 donors)	1 ml	25.2	1.8
donor 1 (normal)	1 ml	18.3	1.8
donor 2 (lipemic)	1 ml	21.0	1.8
donor 3 (lipemic)	1 ml	17.6	1.8
donor 4 (normal)	1 ml	30.5	1.8
donor 5 (normal)	1 ml	20.1	1.8
donor 6 (normal)	1 ml	27.2	1.8
donor 7 (normal)	1 ml	28.9	1.8
donor 8 (normal)	1 ml	25.6	1.8
K562 cells	10^6	35.0	1.9
HeLa cells	10^6	39.1	1.9
Wi38 cells	10^6	4.1	1.8
HCT 116 cells	10^6	45.0	1.9
MDF-7 cells	10^6	11.8	1.9
MDF-7 cells	5×10^6	61.8	1.9

Figure 3:
Agarose gel
analysis
of 32 DNA
samples
isolated from
 10^6 K562 cells;
10 μ l eluate was
used per lane.



Figure 4:
LightCycler
PCR analysis
of 30 DNA
samples
isolated from
 10^6 K562 cells.
5 μ l eluate was
used per PCR.
The curves
show a very low
variation of
Crossing Points.



isolation was started. The MagNA Pure LC automatically performs all isolation and purification steps, such as cell lysis, protein digestion, DNA binding, washing steps, elution of pure DNA, and transfer to a cooled storage cartridge.

Analysis of the isolated DNA

The integrity of the isolated DNA was checked on a 1% agarose gel, together with Molecular Weight Marker III (Roche Applied Science). The DNA yields were calculated from OD_{260nm} measurement, the purity was assessed by calculating the ratio $OD_{260nm}/280nm$. To check the amplificability of the DNA, a LightCycler PCR was performed on all samples using, for example, the Factor V Mutation Detection Kit (Roche Applied Science).

Results and Discussion

DNA integrity

Agarose gel analysis of DNA from blood and cells showed the DNA was of high integrity (Figures 1 and 3). The molecular weight of the isolated DNA was >20 kb.

Reproducibility

Isolation of DNA from 1 ml blood (Figures 1 and 2) or 10^6 culture cells (Figures 3 and 4) in 16-fold to 32-fold replicates showed excellent reproducibility. The coefficient of variation (CV) for yield was $<10\%$, the CV of the LightCycler Crossing Points (CPs) in the PCR was $<3\%$.

Yield and purity

Optical density (OD) analysis revealed yields of up to 30 μ g genomic DNA from 1 ml blood, and up to 62 μ g from culture cells (Table 1). Yields were similar to or higher than, for example, filter tube methods. The $OD_{260nm}/280nm$ ratio was 1.8 ± 0.1 , indicating DNA of high purity. No PCR inhibition was found during the LightCycler analysis.

Cross-contamination

Every second well of the MagNA Pure LC sample cartridge was filled with 1 ml blood (pool), the neighboring wells with buffer (checkerboard pattern). After MagNA Pure LC DNA isolation and subsequent LightCycler PCR analysis (45 cycles), no cross-contamination was found in the 16 samples of buffer, while the 16 blood samples showed the expected signal (data not shown).

Conclusion

The MagNA Pure LC DNA Isolation Kit – Large Volume proved to be a useful and flexible tool for efficient, automated isolation of DNA from various blood volumes and culture cells. All isolation steps are performed automatically by the MagNA Pure LC Instrument within 90–180 minutes, depending on the number of samples and the protocol chosen. The isolated DNA was of high quality and showed no PCR inhibition. Typical yields from 1 ml blood were 17–30 μ g, depending on the donor. Yields from culture cells varied depending on the degree of aneuploidy of the cell line. Yields of up to 62 μ g of DNA could be achieved. The reproducibility was excellent and no cross-contamination was found. Thus the new MagNA Pure LC DNA Isolation Kit – Large Volume provides new applications of the MagNA Pure LC/LightCycler System, and further simplifies nucleic acid analysis. ■

Product	Pack Size	Cat. No.
MagNA Pure LC DNA Isolation Kit – Large Volume	1 kit (96–288 isolations)	3 310 515
MagNA Pure LC Instrument	1 instrument plus accessories	2 236 931

