Roche Applied Science

Application Note MagNA Pure Compact System

No. 01/2005

Two New Protocols for the MagNA Pure Compact System: DNA_Blood_external_lysis and Total_NA_external_lysis

Intended Use of the Instrument

The MagNA Pure Compact Instrument is intended for nucleic acid preparation in general laboratory use by trained professionals.

Purpose of this Note

The MagNA Pure Compact Instrument can rapidly and reliably isolate nucleic acids that are suitable for immediate use in downstream applications such as PCR and RT-PCR. In this Application Note, we compare nucleic acids prepared with two new isolation protocols for the MagNA Pure Compact Instrument (which involve external lysis of samples) to nucleic acids prepared with two existing MagNA Pure Compact Protocols. For these comparison experiments, nucleic acids were isolated from human whole blood, plasma or serum, then analyzed for behavior in PCR analysis on the LightCycler® Instrument and appearance on an electrophoretic gel.

Note: For an overview of the MagNA Pure Compact Instrument and its ability to prepare nucleic acids from unlysed samples of whole blood, cultured cells or serum/plasma, see the following articles in the Biochemica newsletter: *Biochemica* 4 (2003), 11-14; *Biochemica* 3 (2004), 10-11.
For details of MagNA Pure Compact Isolation Procedures, see the MagNA Pure Compact Operator's Manual and the package insert of the MagNA Pure Compact Nucleic Acid Isolation Kit I. To get the latest information on MagNA Pure Compact Protocols available for download, visit our special interest website, www.magnapure.com.

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1. Materials and Methods

Sample Material

For all tests illustrating this Application Note, human blood was used as sample material exemplarily.

- EDTA-preserved whole blood
- plasma prepared from citrate-preserved blood that has been spiked with Parvovirus B19 and Hepatitis A viruses (final concentration of each virus was 10^5 viral copies /ml).
- serum that has been spiked with Parvovirus B19 and Hepatitis A viruses (final concentration of each virus was 10^5 viral copies/ml

External Lysis of Samples

- Various volumes (100, 200 or 400 µl) of whole blood, serum or plasma were transferred into sample tubes.
- 300 µl of lysis/binding buffer (MagNA Pure LC DNA Isolation Kit I – Lysis/Binding Buffer Refill, Cat. No. 03 246 752 001) were added to each sample.
- The samples were mixed thoroughly by pipetting and the lysates were then incubated for 30 min on a roller incubator at room temperature.

Preparation of Nucleic Acids with the MagNA Pure Compact Instrument

Nucleic acids were isolated from each type (externally lysed or unlysed; blood, serum or plasma) and volume (100, 200 or 400 µl) of sample with the MagNA Pure Compact Instrument. Each different type and volume of sample required a separate MagNA Pure Compact Run, which can be summarized as follows:

- In most cases internal control nucleic acids were added.
- Sample tubes, along with appropriate reagent cartridges and disposables, were inserted into the MagNA Pure Compact Instrument.
- The appropriate protocol, sample type, and sample volume for each isolation were selected from the protocol menu on Sample Ordering Screen 2 of the MagNA Pure Compact Instrument.
- For samples that contained internal controls, an internal control volume was specified.
- The MagNA Pure Compact Instrument automatically prepared nucleic acids from each sample and eluted them into appropriate tubes.

Testing of Nucleic Acids

Aliquots were taken from each eluted sample and tested by PCR analysis on the LightCycler® Instrument and gel electrophoresis, as described on the following pages.

Additional Materials Required

LightCycler® Control Kit DNA (Cat. No. 02 158 833)

LightCycler® FastStart DNA Master Hybridization Probes (Cat. No. 03 003 248)

LightCycler® Her2neu DNA Quantification Kit (Cat. No. 03 113 922)

LightCycler® EBV Quantification Kit (Cat. No. 03 330 028)

LightCycler® Hepatitis A Virus Quantification Kit (Cat. No. 03 246 795)

LightCycler® Parvovirus B19 Quantification Kit (Cat. No. 03 246 809)

2. Results Obtained from Whole Blood Using the MagNA Pure Compact DNA_Blood_external_lysis Protocol

LightCycler® Experiment Set-Up

- Internal Control: Control nucleic acid from the LightCycler® EBV Quantification Kit was added to most of the samples to evaluate the internal control function of the MagNA Pure Compact Protocol.
- To compare the purified DNAs prepared with the MagNA Pure Compact DNA_Blood_external_lysis protocol and the DNA_Blood_100_400 protocol, each purified DNA was analyzed by PCR on the LightCycler® System.
- Various targets were amplified with the help of the LightCycler® FastStart DNA Master Hybridization Probes, the LightCycler® Control Kit (which uses Beta-Globin DNA as target) and the LightCycler® Her2neu DNA Quantification Kit.

Isolation Protocol Used	Sample Volume/ Elution Volume (µl)	Results of LightCycler® Instrument Analysis	Amplified Target		
			EBV (Internal Control)	Beta-Globin	Her2neu
DNA_ Blood_ 100_400 (existing protocol)	100/100	number of samples	4	4	4
		CP mean value	34.85	25.83	24.04
		standard deviation	1.12	0.20	0.09
DNA_ Blood_ external_lysis (new protocol)	100/100	number of samples	4	4	4
		CP mean value	32.6	25.50	23.71
		standard deviation	0.54	0.21	0.09

△ **Table 1**: LightCycler® Results with DNA from 100 µl Whole Blood Samples

Isolation Protocol Used	Sample Volume/ Elution Volume (µl)	Results of LightCycler® Instrument Analysis	Amplified Target		
			EBV (Internal Control)	Beta-Globin	Her2neu
DNA_ Blood_ 100_400	200/100	number of samples	8	8	8
		CP mean value	33.55	24.07	22.91
		standard deviation	0.55	0.45	0.36
DNA_ Blood_ external_lysis	200/100	number of samples	8	8	8
		CP mean value	34.00	24.40	23.18
		standard deviation	0.87	0.33	0.44

△ **Table 2:** LightCycler® Results with DNA from 200 µl Whole Blood Samples

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2. Results Obtained from Whole Blood Using the MagNA Pure Compact DNA_Blood_external_lysis Protocol continued

Isolation Protocol Used	Sample Volume/ Elution Volume (µl)	Results of LightCycler® Instrument Analysis	Amplified Target EBV (Internal Control)	Beta-Globin	Her2neu
DNA_ Blood_ 100_400	400/100	number of samples	4	4	4
		CP mean value	32.80	22.40	21.89
		standard deviation	0.19	0.30	0.23
DNA_ Blood_ external_lysis	400/100	number of samples	4	4	4
		CP mean value	33.96	22.75	21.84
		standard deviation	0.59	0.29	0.15

△ **Table 3:** LightCycler® Results with DNA from 400 µl Whole Blood Samples

Isolation Protocol Used	Sample Volume/ Elution Volume (µl)	Results of LightCycler® Instrument Analysis	Amplified Target EBV (Internal Control)	Beta-Globin	Her2neu
DNA_ Blood_ 100_400	400/100 without internal control	number of samples	–	4	4
		CP mean value	–	22.85	22.05
		standard deviation	–	0.04	0.07
DNA_ Blood_ external_lysis	400/100 without internal control	number of samples	–	4	4
		CP mean value	–	23.42	22.63
		standard deviation	–	0.13	0.11

△ **Table 3:** LightCycler® Results with DNA from 400 µl Whole Blood Samples, cont.

Gel Electrophoresis: Experiment Set-Up

Each MagNA Pure Compact Run was used to purify DNA from up to 8 whole blood samples in parallel. Eluted DNA from each sample was characterized by agarose gel electrophoresis (1% agarose gel). DNA Molecular Weight Marker III was included on each gel for comparison purposes.

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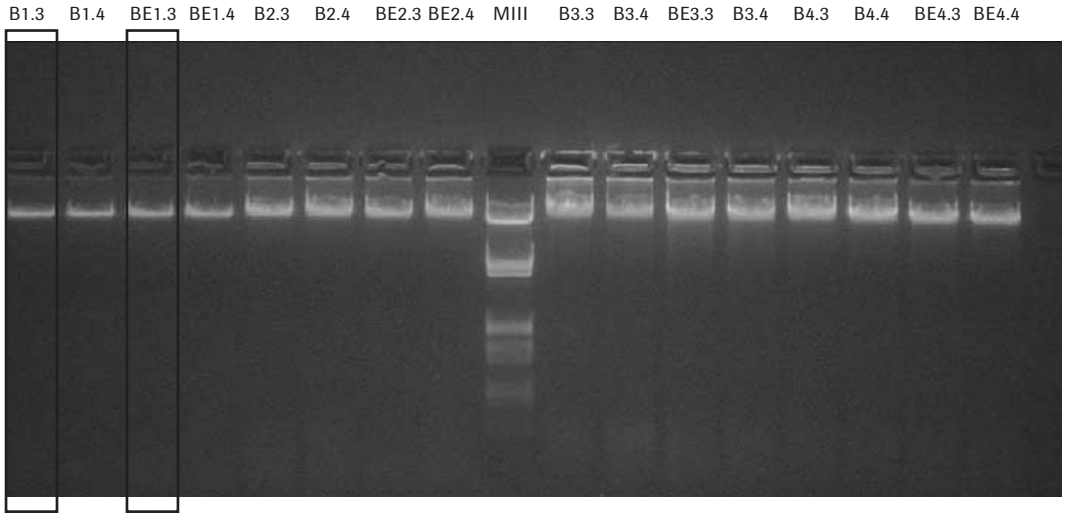
2. Results Obtained from Whole Blood Using the MagNA Pure Compact DNA_Blood_external_lysis Protocol continued

Gel Electrophoresis: Results

Identification Code for MagNA Pure Compact Runs and Gel Lanes	Lane Contains DNA Obtained with this MagNA Pure Compact Isolation Protocol	Sample Volume (µl)
B1	DNA_Blood_100_400	100
BE1	DNA_Blood_external_lysis	100
B2	DNA_Blood_100_400	200
BE2	DNA_Blood_external_lysis	200
B3	DNA_Blood_100_400	400
BE3	DNA_Blood_external_lysis	400
B4	DNA_Blood_100_400*	400
BE4	DNA_Blood_external_lysis*	400

*instrument run without internal control function

Samples on the gel are identified by a code. For example, lane BE1.3 contains DNA purified from sample #3 (volume, 100 µl) of isolation run BE1, which used the DNA_Blood_external_lysis protocol.



△ **Figure 1:** Comparison of high molecular DNA obtained with the two "whole blood" protocols (gel 1 of 3). For example, lane **B1.3** (DNA obtained with the old DNA_Blood_100_400 protocol) has a band pattern identical to lane **BE1.3** (DNA obtained with the new DNA_Blood_external_lysis protocol).

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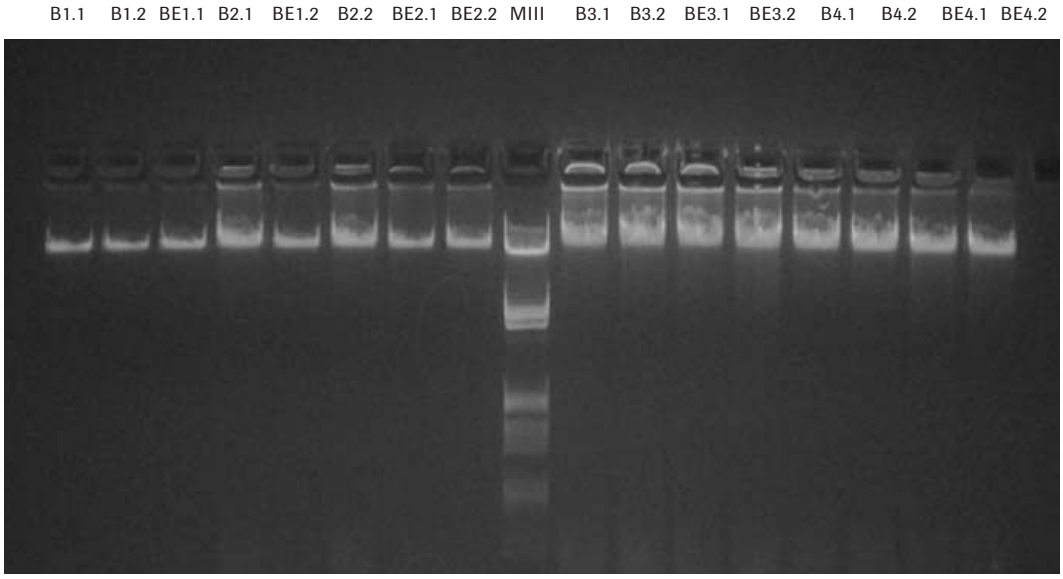
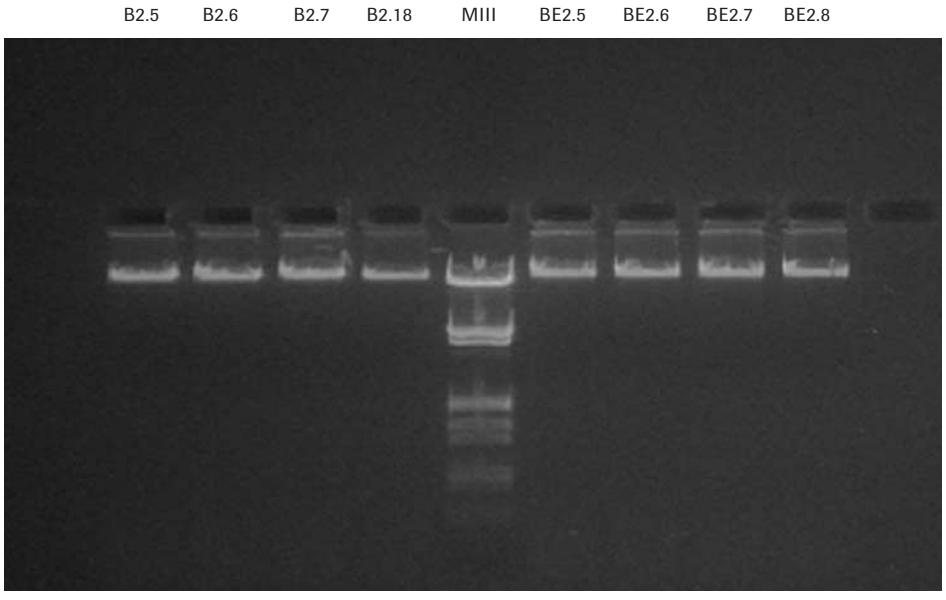
2. Results Obtained from Whole Blood Using the MagNA Pure Compact DNA_Blood_external_lysis Protocol continued



△ **Figure 2:** Comparison of high molecular DNA obtained with the two “whole blood” protocols (gel 2 of 3).



△ **Figure 3:** Comparison of high molecular DNA obtained with the two “whole blood” protocols (gel 3 of 3).

3. Results Obtained from Plasma and Serum Using the MagNA Pure Compact Total_NA_Plasma_external_lysis Protocol

LightCycler® Experiment Set-Up

- Internal Control: Control nucleic acids (NAs) from the LightCycler® Hepatitis A Virus Quantification Kit and LightCycler® Parvovirus B19 Quantification Kit were spiked into most of the MagNA Pure Compact Samples to evaluate the internal control function of the MagNA Pure Compact Protocol.

  Note: Since the target virus competes with the internal control NA, amplification of a control NA does not always lead to a detectable signal. Therefore, a mean value and standard deviation for the internal controls are only shown for some samples, *e.g.* the 100 µl plasma samples (in Table 4 below).

- To compare NAs prepared with the Total_NA_Plasma_external_lysis protocol and the Total_NA_Plasma_100_400 protocol, each purified NA was analyzed by PCR on the LightCycler® Instrument. Various targets were amplified with the help of the LightCycler® Hepatitis A Virus Quantification Kit and LightCycler® Parvovirus B19 Quantification Kit.

Protocol Used	Sample Volume/ Elution Volume (µl) / Insrument	Results of LightCycler® Analysis	Amplified Target: HAV	HAV Internal Control	Parvo B19	ParvoB19 Internal Control
Total_NA Plasma_ 100_400	100/50 instrument 1	number of samples	4	4	4	4
		CP mean value	33.41	34.30	28.14	32.49
		standard deviation	1.03	0.48	0.27	0.94
Total_NA Plasma_ external_lysis	100/50 instrument 1	number of samples	4	4	4	4
		CP mean value	33.04	35.24	27.89	35.56
		standard deviation	0.41	0.72	0.03	1.62
Total_NA Plasma_ external_lysis	100/50 instrument 2	number of samples	4	4	4	4
		CP mean value	31.64	33.87	27.61	33.72
		standard deviation	0.53	0.76	0.13	2.65

△ **Table 4:** LightCycler® Results with NA from 100 µl Plasma Samples

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3. Results Obtained from Plasma and Serum Using the MagNA Pure Compact Total_NA_Plasma_external_lysis Protocol continued

Protocol Used	Sample Volume/ Elution Volume (µl) / Insrument	Results of LightCycler® Analysis	Amplified Target HAV	Amplified Target ParvoB19
Total_NA Plasma_ 100_400	200/50 instrument 1	number of samples	8	8
		CP mean value	33.43	26.79
		standard deviation	0.77	0.09
Total_NA Plasma_ external_lysis	200/50 instrument 1	number of samples	8	8
		CP mean value	32.69	26.78
		standard deviation	0.47	0.19
Total_NA Plasma_ external_lysis	200/50 instrument 2	number of samples	8	8
		CP mean value	30.00	26.58
		standard deviation	0.28	0.07

△ **Table 5:** LightCycler® Results with NA from 200 µl Plasma Samples

Protocol Used	Sample Volume/ Elution Volume (µl) / Insrument	Results of LightCycler® Analysis	Amplified Target HAV	Amplified Target ParvoB19
Total_NA Plasma_ 100_400	400/50 instrument 1	number of samples	4	4
		CP mean value	32.41	25.58
		standard deviation	1.21	0.19
Total_NA Plasma_ external_lysis	400/50 instrument 1	number of samples	4	4
		CP mean value	31.83	25.87
		standard deviation	0.81	0.23
Total_NA Plasma_ external_lysis	400/50 instrument 2	number of samples	4	4
		CP mean value	29.62	25.56
		standard deviation	0.21	0.16

△ **Table 6:** LightCycler® Results with NA from 400 µl Plasma Samples

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3. Results Obtained from Plasma and Serum Using the MagNA Pure Compact Total_NA_Plasma_external_lysis Protocol continued

Protocol Used	Sample Volume/ Elution Volume (µl) / Insrument	Results of LightCycler® Analysis	Amplified Target HAV	Amplified Target ParvoB19
Total_NA Plasma_ 100_400	400/100 without internal control instrument 1	number of samples	4	4
		CP mean value	30.64	26.06
		standard deviation	0.61	0.08
Total_NA Plasma_ external_lysis	400/100 without internal control instrument 1	number of samples	4	4
		CP mean value	30.14	26.44
		standard deviation	0.27	0.26
Total_NA Plasma_ external_lysis	400/100 without internal control instrument 2	number of samples	4	4
		CP mean value	30.44	26.80
		standard deviation	0.54	0.28

△ **Table 6:** LightCycler® Results with NA from 400 µl Plasma Samples

Protocol Used	Sample Volume/ Elution Volume (µl) / Insrument	Results of LightCycler® Analysis	Amplified Target HAV	Amplified Target ParvoB19
Total_NA Plasma_ external_lysis	400/100 instrument 1	number of samples	4	4
		CP mean value	30.2	26.42
		standard deviation	0.29	0.30
Total_NA Plasma_ external_lysis	400/100 instrument 2	number of samples	4	4
		CP mean value	30.24	26.17
		standard deviation	0.30	0.04

△ **Table 7:** LightCycler® Result with NA from 400 µl Serum Samples*

* **Note:** Compare the results from these 400 µl serum samples to results from the 400 µl plasma samples purified with both the old and new protocols (in Table 6 above).

4. Conclusion and Acknowledgment

Conclusion

The LightCycler® Results and gel electrophoresis experiments shown in sections 2 and 3 of this Note demonstrate that the new MagNA Pure Compact DNA_Blood_external_lysis and Total_NA_external_lysis protocols produce purified nucleic acids that perform comparably to nucleic acids purified with the existing DNA_Blood_100_400 and Total_NA_Plasma_100_400 protocols.

Acknowledgment

The data in this note were provided by Dr. Anja Eiblmaier, MicroCoat, Bernried, Germany.

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