For general laboratory use.

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MagNA Pure Compact Nucleic Acid Isolation Kit I – Large Volume

Version: 10
Content version: August 2015

Prefilled reagents for the isolation of genomic DNA from mammalian whole blood, cultured cells or tissue [1], and total nucleic acids from mammalian serum or plasma, using the MagNA Pure Compact Instrument.

1) Only in combination with MagNA Pure DNA Tissue Lysis Buffer (Cat. No. 04 805 160 001)

Cat. No. 03 730 972 001	1 kit 32 isolations

Store the kit at +15 to +25°C

1. General Information

1.1. Contents

Label	Function	Content
Reagent Cartridge	Contains reagents sufficient for one nucleic acid isolation run (well 1: Proteinase K; wells 2,3: Lysis Buffer; wells 4,5: MGPs; well 6: isopropanol; wells 7,8: Wash Buffer I; well 9: Wash Buffer II; well 10: Wash Buffer III; well 11: Elution Buffer).	32 sealed cartridges
Tip Tray	Contains Reaction Tips (2 large and 1 small) and Piercing Tool.	32 disposable Tip Trays
Sample Tube	To be placed into the Tube Rack of the MagNA Pure Compact Instrument (see Operator's Manual).	35 tubes, 2.0 ml
Elution Tube	To be placed into the Elution Tube Rack of the MagNA Pure Compact Instrument (see Operator's Manual).	35 barcoded tubes, 2.0 ml
Elution Tube Cap	To seal the Elution Tubes.	35 tube caps

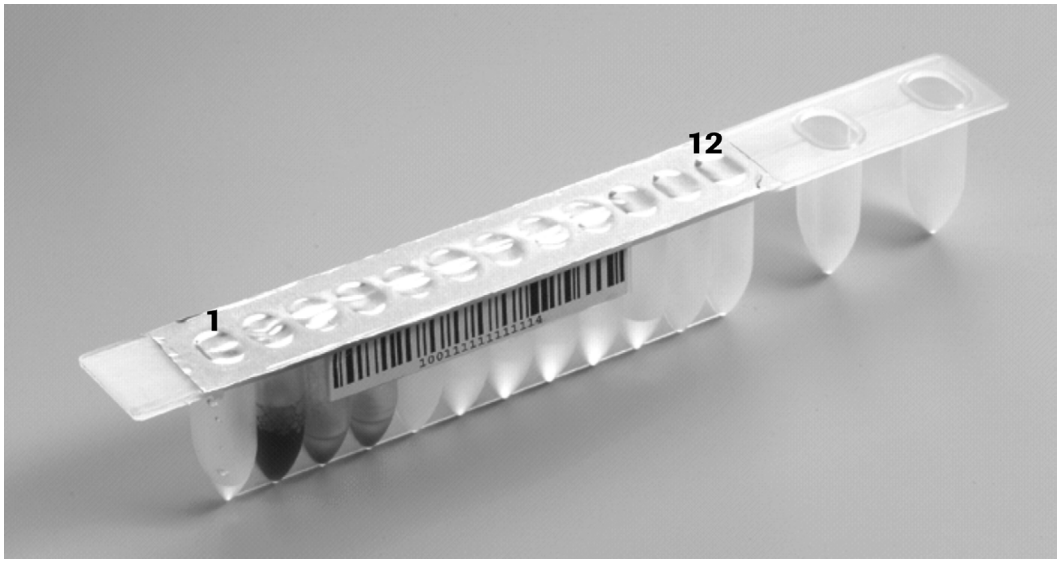

Fig. 1: Reagent Cartridge of the MagNA Pure Compact Nucleic Acid Isolation Kit I Large Volume

1.2. Storage and Stability

Storage Conditions (Product)

The kit is shipped at ambient temperature.

The unopened kit components of the MagNA Pure Compact Nucleic Acid Isolation Kit – Large Volume are stable at +15 to +25°C until the expiration date printed on the label.

1.3. Additional Equipment and Reagents Required

Additional reagents and equipment required to perform nucleic acid isolations with the MagNA Pure Compact Nucleic Acid Isolation Kit I - Large Volume using the MagNA Pure Compact Instrument:

- Standard laboratory equipment
- Pipettes and nuclease free, aerosol-preventive tips to pre-dispense samples into the Sample Tubes
- centrifuge and suitable tubes
- optional, when using Internal Control:
  2.0 ml Sarstedt Tubes (without cap: Sarstedt #72.608; with cap: Sarstedt #72.693)
- optional for isolation of genomic DNA from tissue:
  MagNA Pure DNA Tissue Lysis Buffer*

1.4. Application

The MagNA Pure Compact Nucleic Acid Isolation Kit I - Large Volume, a General Purpose Reagent (GPR), is specifically designed to isolate highly purified genomic DNA from mammalian whole blood or cultured cells, and total nucleic acids (e.g., viral DNA and RNA) from mammalian serum or plasma, using the MagNA Pure Compact Instrument. In combination with the MagNA Pure DNA Tissue Lysis Buffer and the DNA_Tissue purification protocol, genomic can be isolated from 1 – 10 mg of mammalian tissue. For a detailed pretreatment protocol, please refer to the online available Instructions for Use for the MagNA Pure DNA Tissue Lysis Buffer.
The purified nucleic acids can be used in PCR or RT-PCR on the LightCycler® Instruments, standard thermal block cyclers, or in other typical downstream applications. Purified nucleic acid is free of any PCR inhibitors.

The MagNA Pure Compact Nucleic Acid Isolation Kit I - Large Volume cannot be used for co-isolation of RNA from mammalian whole blood or cultured cells.

The isolation reagents are provided in pre-filled, sealed, and barcoded MagNA Pure Compact Reagent Cartridges. Each cartridge contains all reagents required for a single isolation. MagNA Pure Compact Reaction tips are provided in a disposable MagNA Pure Compact Tip Tray. MagNA Pure Compact Tubes for samples and purified nucleic acid eluates are barcoded. The kit is designed for 32 isolations (4 × 8) from:

- 500 or 1,000 µl mammalian whole blood (containing no more than 1 × 10^4 cells/µl)
- 500 or 1,000 µl mammalian serum or plasma
- 100 µl suspension of cultured cells (containing no more than 2 × 10^6 cells)

If combined with MagNA Pure DNA Tissue Lysis Buffer :

- 1-10 mg of mammalian tissue (for a detailed protocol, please refer to the online available Instructions for Use for the MagNA Pure DNA Tissue Lysis Buffer)

1.5. Preparation Time

- Setup: 15 minutes
- Total time to purify 8 blood samples: 25 minutes

No hands-on time is required after setup.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

To obtain optimal results in downstream procedures, especially in real-time PCR assays on the LightCycler® instruments, **do not process samples with higher volume or cell count than the selected purification protocol is designed to handle!** Doing so will affect the performance of the isolation process, may lead to clumping and loss of MGPs, cross contamination of samples, or even damage of the instrument.

⚠ ***Treat all samples as potentially infectious.***

Optimal Amounts of Sample Material

- **500 or 1,000 µl of mammalian whole blood, containing no more than 1 × 10^4 WBCs/µl)**

⚠ ***If you use lower volumes of whole blood samples, fill up with PBS to 500 µl or 1,000 µl, respectively, and mix well.***

⚠ ***We strongly recommend to count the number of WBCs in whole blood samples manually with a hemocytometer (such as a Neubauer device with counting chambers) before processing them. Automatic counting systems (depending on the supplier) sometimes produce cell counts that do not agree with manual hemocytometer counts. All cell numbers given in this protocol were determined manually with a standard hemocytometer.***

⚠ ***The purification protocols for whole blood were established with human blood samples from healthy individuals. Remember that unhealthy or drug-treated individuals may show abnormal blood qualities that may influence the nucleic acid isolation process.***

⚠ ***Different mammalian species may have different concentrations of blood cells. For some species, you may need to use a smaller sample of blood to keep the cell numbers within the above guidelines.***

⚠ ***Use only whole blood containing anticoagulants. Avoid using samples that were stored at +15 to +25°C. Whole blood may be stored at +15 to +25°C for a maximum of one day, or at +2 to +8°C for one week. For longer storage times, whole blood samples should be frozen, otherwise this can result in a slow loss of DNA yield and integrity.***

- **500 µl or 1,000 µl of mammalian serum or plasma**
- **Up to 2 × 10^6 cultured cells in a volume of 100 µl**

ⓘ *Centrifuge the cells to remove culture medium, then resuspend the cell pellet in 100 µl PBS.*

⚠ ***The DNA content of different cell lines may vary to a large extent due to different degrees of aneuploidy. Reduce the number of input cells for cell lines with an extremely high DNA content such as K562 or HeLa cells to avoid clumping. For best results, start with 2 to 5 × 10^5 cells.***

In combination with MagNA Pure DNA Tissue Lysis Buffer the following amounts of sample material can be used:

- **1 – 10 mg fresh, fresh-frozen or unfrozen, stabilized (e.g. treated with RNAlater) mammalian tissue**

ⓘ *Pretreatment procedures for different sample materials are described in the Instructions for Use of the MagNA Pure DNA Tissue Lysis Buffer.*

Control Reactions

Always run appropriate controls with the samples, especially if you want to perform quantification analyses of the eluted nucleic acid samples (*e.g.,* by real-time PCR assays on the LightCycler® Instrument). In order to control the complete process starting from sample preparation to quantification analysis, perform the following controls:

- Positive Control, by using a sample material positive for your target.
- Negative Control, by using a sample material negative for your target.
- Internal Control (IC), by adding a defined amount of a control template to all samples to be purified.

The Internal Control (IC) is added prior to the purification step and then co-purified and amplified with your target of interest from the specimen in the same PCR reaction. The IC concept is especially useful for enzyme-based amplification processes such as PCR, because efficiency of the PCR process might be reduced by inhibitors present in the purified sample material. In addition, the Internal Control is used to compensate for possible losses of your target during purification.

For PCR quantification assays on the LightCycler® instruments, use a synthetic double-stranded DNA molecule with primer-binding sites identical to those of your target sequence, but having a unique probe-binding region that differentiates the IC from the target-specific amplicon. For RT-PCR assays use an in vitro transcribed RNA molecule with the same features as above. Discriminate the signals derived from your target and the IC by performing a dual-color HybProbe assay. For detailed information regarding the IC concept in combination with the LightCycler® Carousel-Based System, read LightCycler® Technical Note 12/2000 "Absolute Quantification with External Standards and an Internal Control" available at www.lightcycler.com.

Internal Control

The MagNA Pure Compact Instrument offers the option to add an Internal Control (IC) to the samples during the purification procedure.

⚠ ***Including an Internal Control is important to detect a possible impairment of the nucleic acid isolation process.***

Two Possible Methods for Incorporating the IC

1. IC is added directly to the Sample

- On Sample Ordering Screen I, set "Internal Control Volume" to "none". Document the use of the IC in the "Comment" field.
- Use this method for suspensions of cultured cells in PBS.

⚠ ***Do not use this method when the sample is blood or plasma/serum and the IC is naked DNA or RNA, because the IC might be degraded by nucleases present in these sample materials.***

2. IC is provided in the Sample Tube Rack

- Pipet the IC (5, 10, or 20 µl) into a separate tube. Use only the tubes specified in "Additional Equipment and Reagents Required".
- Place each tube in the assigned position in row 2 of the Tube Rack.
- On Sample Ordering Screen I, enter the amount of IC used.
- The instrument automatically incorporates the IC into the isolation process, mixes it with lysis buffer, thereby, protecting it from degradation by nucleases.
- Use this method for whole blood, serum, or plasma samples. Naked DNA or RNA can be used as IC.

⚠ ***If you chose the IC option in the software and you do not place an IC tube in the Tube Rack, lysis buffer will be pipetted onto the instrument stage! Cleaning of the instrument will be necessary. If your MagNA Pure Compact Instrument uses software version 1.1, the lysis buffer might come into contact with the sample before it is pipetted into the IC tube. In this case, cleaning and decontamination of the instrument is necessary.***

General Considerations

Handling Requirements

The isolation procedure is designed to process 8 samples at the same time. The instrument can handle all numbers of samples between 1 and 8. For detailed description of instrument setup and handling refer to the MagNA Pure Compact Operator's Manual.

⚠ ***Adapt the Reagent Cartridge to room temperature (+15 to +25°C) before use. If you use the reagents at temperatures outside the recommended range, the kit may not work well.***

⚠ ***To ensure correct pipetting, use only the Sample Tubes contained in the kit and the recommended types of tubes for the Internal Control. (See "Additional Equipment and Reagents Required")***

⚠ ***Document the kit lot no. in case of complaints or questions for Roche technical services, regarding any component of the kit (reagent cartridges or disposables).***

⚠ ***Make sure you have followed the instructions regarding type and amount of sample material. Wrong type and amount of sample material may cause clumping of MGPs which cannot be detected by the Clot Detection function of the instrument. Clumping of MGPs may lead to low yield and purity of nucleic acids, cross contamination, and inhibition of down-stream assays (*e.g.,* PCR).***

⚠ ***After the run has finished, carefully inspect the instrument for any signs of spillage. If spillage occured, clean the instrument as described in the Operator's Manual.***

⚠ ***Some buffers (wells 2, 3, 7 and 8) contain guanidinium salts which are hazardous irritants. Do not allow to come in contact with skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If necessary, immediately contact your laboratory supervisor, and seek medical assistance. When spilling these reagents, dilute the spill with large amounts of water before attempting to clean up the spill.***

⚠ ***Do not allow the above two buffers to mix with sodium hypochlorite (bleach) solution or strong acids. This mixture can produce a highly toxic gas.***

Prevention of cross contamination

- To minimize the risk of cross contamination and to prevent contact with potentially infectious materials, always complete the processes involving sample preparation such pipetting of blood samples in a designated part of the benchtop or safety hood before starting PCR setup.
- To further minimize the risk of contamination, always carry out sample preparation, PCR setup and the PCR run itself in separate rooms specially designated for each phase in the workflow.

Safety Information

Laboratory procedures

Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis/Binding Buffer or take appropriate measures, according to local safety regulations.

- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.
- Complete each phase of the PCR/RT-PCR workflow before proceeding to the next phase. For example, you should finish PCR/RT-PCR sample preparation before starting PCR/RT-PCR set-up. Sample preparation, PCR/RT-PCR set-up and the PCR/RT-PCR run itself should also be performed in separate locations.

Waste Handling

- Discard unused reagents and waste in accordance with country, federal, state and local regulations.
- Please folow the instructions in the Safety Data Sheets (SDS).

2.2. Protocols

To perform DNA isolation from mammalian blood or cultured cells, or total nucleic acid isolation from mammalian serum/plasma samples with the MagNA Pure Compact Nucleic Acid Isolation Kit I - Large Volume, 5 different pre-installed purification protocols are available. For each protocol, sample and elution volumes must be chosen from the software menu.

New protocols or protocol updates can be downloaded from http://www.magnapure.com. Read the instructions for downloading and installing the purification protocol carefully. For additional details, contact your local Roche representative.

Use the following table to decide which purification protocol is best for your sample material:

⚠ ***The different protocols are optimized for a specific sample material each. Do not use a protocol for a sample material other than specified below! Doing so will affect the performance of the isolation process, may lead to clumping and loss of MGPs, cross contamination of samples, or even damage of the instrument.***

Onboard MagNA Pure Compact software programs for six purification protocols currently available for use with the MagNA Pure Compact Nucleic Acid Isolation Kit I – Large Volume:

Protocol Name	Sample Material	Optional Internal Control [1)]	Elution Volume [3)]
DNA_Blood_1000	1,000 µl whole blood (≤ 1 × 10^7 cells)	yes	200 µl
DNA_Blood_500	500 µl whole blood (≤ 5 × 10^6 cells)	yes	100 or 200 µl
Total_NA_Plasma_1000	1,000 µl serum/plasma	yes	50 or 100 µl
Total_NA_Plasma_500	500 µl serum/plasma	yes	50 or 100 µl
DNA_Culture_Cells_LV	100 µl cell suspension (≤ 2 × 10^6 cells)	no [2)]	200 µl
DNA_Tissue	200 µl lysate from 1 - 10 mg mammalian tissue	no	200 µl

1) See “Internal Control” for detailed information.
2) The DNA_Culture_Cells_LV protocol does not allow to use an Internal Control. If an Internal Control is required in combination with this protocol, pipet approx. 20 µl Internal Control directly into the cell suspension (the Internal Control is stable in PBS).
3) The concentration of nucleic acids in the eluate, and therefore sensitivity in downstream applications, can be increased by choosing a low elution volume; however, elution efficiency and overall nucleic acid yield will be approx. 10 - 30% less compared to the high elution volume (*e.g.,* the typical DNA yield from 500 µl human whole blood using an elution volume of 100 µl is 12.9 mg, compared to 15.8 mg when using 200 µl elution volume). For DNA isolation from cultured cells or 1 ml whole blood, this variable elution volume option is not included due to the very high DNA content of this sample material.

⚠ ***Only one protocol (and thus one sample material) can be selected for each run. Therefore, different types of sample material (or different sample or elution volumes) cannot be combined in the same run.***

Protocol for the Isolation of Nucleic Acids

1. Turn on the instrument.
   Remove Cartridge Rack and Tube Rack (with Elution Tube Rack) from the instrument.
   Click the Run button on the Main Menu Screen to access Sample Ordering Screen 1.
   Follow the software-guided workflow.

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2. Remove a pre-filled Reagent Cartridge from the blister pack.
   ⚠ ***Check the cartridge integrity and filling volumes of the wells. Do not use cartridges that have a different pattern of filling or that are damaged.***
   Adapt the Reagent Cartridge to +15 to +25°C (30 min).
   ⚠ ***Handle each Reagent Cartridge prior to use as follows:***
   –Always wear gloves when handling the MagNA Pure Compact cartridge.
   –Hold the cartridge only at the barcode imprinted area and the opposite side.
   –Avoid touching the sealing foil covering the cartridge wells.
   –Avoid touching the two single open wells and do not use them as handles.
   –Avoid any foam formation. Let the fluid within the cartridge wells settle again completely. If fluid remains under the sealing foil, knock the cartridge bottom gently on a flat lab bench surface. This is especially important for well 1 which contains a small volume of proteinase K.

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3. Scan the barcode.
   With the two isolated wells pointing away from you, insert all the wells on the Reagent Cartridge into the holes in the Cartridge Rack. Use the guide slots on the rack to help position the cartridge.
   Repeat the steps above for the desired numbers of samples (1 to 8).

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4. Proceed to Sample Ordering Screen 2.
   Select the appropriate purification protocol from the Protocol menu
   Select the elution volume (50 µl, 100 µl, or 200 µl).
   Optional: Select the Internal Control Volume (5 µl, 10 µl, or 20 µl).

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5. Insert the appropriate number of Tip Trays (one per purification) into the assigned position in the instrument Tip Rack.
   ⚠ ***Check if the tip tray holds a disposable in each position (tip or piercing tool). Do not use tip trays that are not assembled accordingly.***
   ⚠ ***Handle Tip Trays with care to prevent tips or piercing tool from falling out of the tray. Should this happen, discard the respective tip tray and tips. Use the Tip Tray Kit to replace missing Tip Trays.***
   Proceed to Sample Ordering Screen 3.

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6. Scan the sample barcode from the primary sample tube or enter the sample name.
   Arrange the Sample Tubes in row 1 of the Tube Rack. Make sure the brim of the tubes seats solidly on the rack.
   Pipet the samples into their respective Sample Tubes.
   Proceed to Sample Ordering Screen 4.
   ⚠ ***This screen only appears and accepts information about Internal Control Tubes if you selected a protocol with internal controls on Sample Ordering Screen 2. The program will skip this screen and proceed directly to Sample Ordering Screen 5 if you selected a protocol with no IC.***

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7. Pipet the proper amount of IC (as specified on Sample Ordering Screen 2) into one of the IC Tubes.
   Identify the tube by attaching your own barcode label or writing an ID number on the tube with a permanent marker.
   Put the filled control tubes in row 2 of the Tube Rack.
   Touch IC Sample ID field 1. In that field, enter the ID of Control 1 (i.e., the one you just put in the rack) by either scanning the (self-attached) barcode or using the virtual keyboard to type the ID number. (Touch the Keyboard button to access the keyboard.)
   Repeat the above control placement and identification steps for each control used.
   Insert the Tube Rack with sample and ICs.
   Touch the “IC Tubes inserted” confirmation button.

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8. Reinsert the Tube Rack into the instrument.
   Proceed to Sample Ordering Screen 5.

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9. Scan the bar codes of the Elution Tubes.

Place the Elution Tubes into the Elution Tube Rack. Make sure the brim of the tubes seats solidly on the rack. Reinsert the Elution Tube Rack into the instrument.

10 On the Confirmation Screen, check the information display.
If the information is correct, confirm it by touching the “Confirm Data” button, close the front cover, and start the run.

11 After the purification run has ended, the Result Screen appears showing the result of the isolation process for each channel:
–The result will be PASS if the isolation run was completed without any warning or error.
–The result will be FAIL if any interruption of the process or error occurred during the run. For each FAIL result, the result screen will show a brief error or warning messages to help you decide whether the error or warning can be ignored. Refer to the troubleshooting section of the MagNA Pure Compact Operator’s Manual.

12 Close the Elution Tubes with the supplied tube caps and remove the Elution Tube Rack or the Elution Tubes immediately after the end of the purification run.
If not proceeding directly to your downstream application, store eluates at −15 to −20°C (if target nucleic acid is DNA) to −70°C (if large nucleic acid is RNA). Nucleic acids are stable for at least 6 to 12 months if stored properly.

13 Optionally: Start the automated liquid waste discard. Empty the MagNA Pure Compact Waste Tank after waste handling. Treat liquid waste as potentially infectious (depending on sample material), and hazardous, since lysis buffers are present (see Safety Information).

3. Results

DNA Yield and DNA Purity

DNA was isolated from normal human whole blood samples or from K562 cultured cells, and eluted in 200 µl Elution Buffer. Yields were determined by OD_{260nm} measurement. Purity was determined by $OD_{260/280}$ measurement. For typical results see table below.

Sample Material	Volume/Amount	DNA Yield (µg)	$OD_{260/280}$ Ratio
Whole Blood	500 µl	14.1	≥1.7
Whole Blood	1,000 µl	30.1	≥1.7
K562 cells	1 x 10^6	40.4	≥1.7
K562 cells	2 x 10^6	47.6	≥1.7

The DNA yield from whole blood strongly depends on the specific blood donor and blood cell count. The yield from cultured cells depends on the cell line due to the variable degree of aneuploidy. K562 cells for example have a high DNA content, therefore it is better to start from only 1 × 10^6 cells.

Plasma/serum does not contain enough DNA to measured the DNA yield by OD_{260} nm.

DNA Integrity

DNA was isolated in replicates of 4 from 1,000 µl human whole blood taken from two different donors. Elution volume was set to 200 µl. Integrity of DNA was shown by subjecting each eluate to agarose gel electrophoresis together with a suitable molecular weight marker such as *Molecular Weight Marker III*. All samples revealed bands of genomic DNA with a size of at least 20 kb.

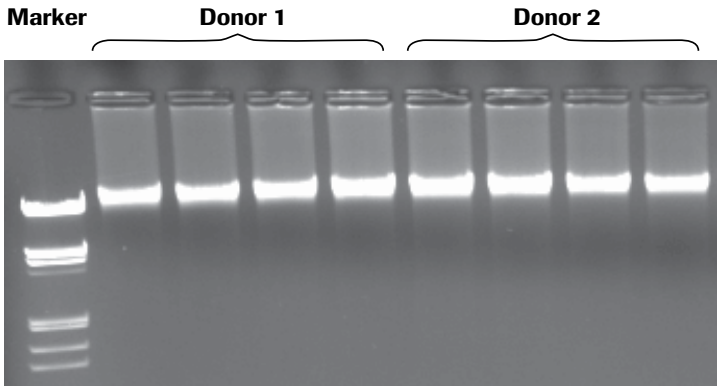

Fig. 2: 2.5 µl DNA isolated from 1,000 µl human whole blood analyzed by agarose gel electrophoresis

Sensitivity in Real-time PCR Applications

Citrate-preserved plasma was spiked with a dilution series of human Parvo B19 Virus in the range of 10^2 to 10^7 copies/ml. A one milliliter sample of spiked plasma was used for nucleic acid isolation. With the elution volume set to 100 µl, five microliters of sample eluate were used in a LightCycler® HybProbe PCR assay targeting Parvo B19 virus. As lfew as 500 copies/ml (= 5 copies per PCR reaction) were routninely detected, clearly demonstrating the excellent sensitivity and linearity of the MagNA Pure Compact nucleic acid isolation procedure.

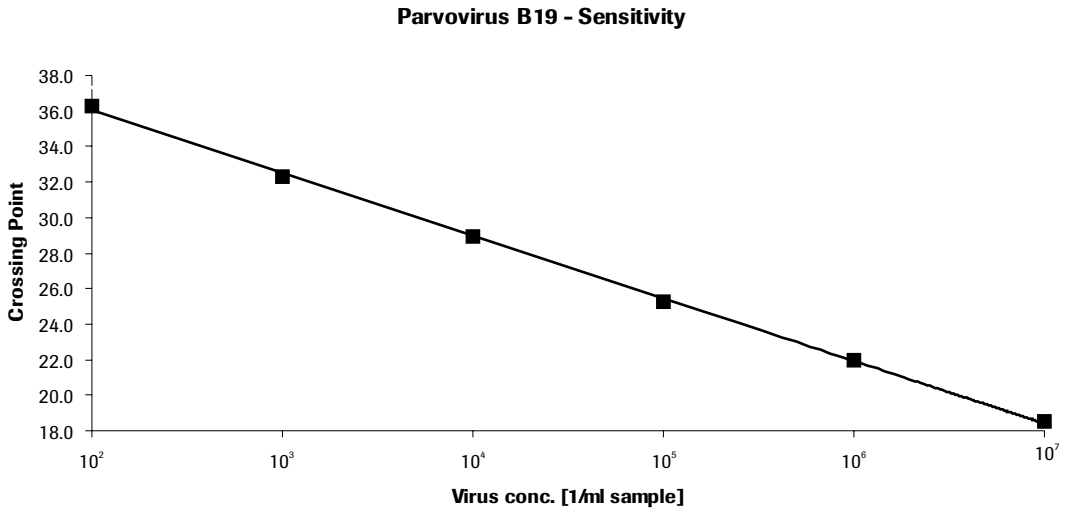

Fig. 3: LightCycler® HybProbe analysis (targeting Parvo B19 Virus) of DNA samples isolated from whole blood samples spiked with different concentrations of Parvo B19 Virus

4. Troubleshooting

Observation	Possible cause	Recommendation
Clumping of beads/ problem with magnetic separation of beads	Too much sample material.	Reduce amount of sample material to the values recommended in section “Sample Materials”.
	MGPs were magnetized prior to use	Avoid contact between MGPs and magnets. Store kit appropriately.
Carryover of MGPs into eluate	Too much sample material was used causing clumping of MGPs.	Reduce amount of sample material to the values recommended in section “Sample Materials”.
		Centrifuge Elution Tube to sediment MGPs. Transfer MGP-free supernatant to a fresh tube.
Nucleic acid is degraded	Storage of samples was not optimal.	Use fresh or frozen samples. Avoid using samples that were stored at room temperature for extended periods of time.
	Storage of eluates was not optimal.	Do not store eluates at +15 to +25°C.
		Do not perform overnight runs.
	Nuclease contamination of Reaction Tips, Sample Tubes, Elution Tubes, or reagents	Avoid contaminating disposables and reagents with nucleases.
Poor nucleic acid yield	Sample did not contain enough cells.	Cultured cells: Count cells before use. Optimal results are obtained with 1 × 10^6 cells.
		Blood: Do not use clotted or sedimented blood. Use fresh or frozen blood containing anticoagulants. Mix tubes before use.
	Storage of samples was not optimal.	see above
	Too much sample material was used causing clumping of MGPs.	Reduce amount of sample material to the values recommended in section “Sample Materials”.
	Reagents were in wrong position on stage.	Make sure that all reagents are in their correct positions on the stage.
Poor nucleic acid purit	Too much sample material.	Reduce amount of sample material to the values recommended in section “Sample Materials”.
	Storage of samples was not optimal.	see above
Cross-contamination of samples	Too much sample material was used	Reduce amount of sample material to the values recommended in section “Sample Materials”.
Poor performance in downstream assays (PCR/RT-PCR)	Concentration of template DNA was not optimal.	Check DNA concentration in the eluted DNA samples and adjust input amount of eluate per PCR.
		Optimum: 10 - 100 ng DNA/PCR.
		Optimize amount of input sample material. Refer to the section “Sample Materials”.
	Poor purity of DNA.	Too much sample material. Refer to the section “Sample Materials”.
	Storage of samples was not optimal	see above
	Improper storage of eluate.	see above
	(RT-) PCR reagents or protocols were not optimal.	Check (RT-)PCR reagents and protocols with a positive DNA or RNA control *(e.g.,* Human Genomic DNA) or use an Internal Control.

Eluates show slightly red color	Minimal abrasion from magnetic particles.	Centrifuge at low *g*-values (approx. 1,000 rpm) to remove fines. *The red color does not affect subsequent (RT)-PCR assays on the LightCycler® Instruments or conventional thermal block cycler.*
Low yields of elution volume	In some cases only a certain portion of the eluted material is transferred to the elution tubes	The quality of the isolated nucleic acid is not impacted. We recommend transferring that portion of the eluate still remaining in the reagent cartridge manually to a vial either for storage or a subsequent application, such as PCR.

5. Additional Information on this Product

5.1. Test Principle

How this Product Works

MagNA Pure Compact Nucleic Acid Isolation Kit I – Large Volume is used with the MagNA Pure Compact Instrument to purify high-quality, undegraded genomic DNA from mammalian whole blood or cultured cells, and total nucleic acids (*e.g.,* viral DNA and RNA) from mammalian serum or plasma. The isolated DNA can be eluted into 50, 100, or 200 µl (depending on the purification protocol used). It meets the quality standards required for highly sensitive and quantitative PCR or RT-PCR analysis on the LightCycler® Instruments.
The MagNA Pure Compact System consists of the instrument, reagents and disposables:

- The instrument can perform 1 – 8 isolations of DNA per run.
- The isolation reagents are provided in pre-filled, sealed Reagent Cartridges. Each cartridge contains all reagents required for one isolation.
- The reaction tips needed for each isolation are provided in a disposable Tip Tray. Also, barcoded and sterile tubes for uptake of samples and nucleic acid eluates are supplied.

After instrument setup and starting the software-guided isolation protocol, the MagNA Pure Compact Instrument performs all isolation steps automatically.

Test Principle

The nucleic acid isolation procedure of the MagNA Pure Compact Instrument and the MagNA Pure LC Instruments is based on the proven MagNA Pure magnetic-bead technology. The principal steps of the MagNA Pure Compact nucleic acid isolation procedure are:

① The samples are lysed by incubation with Proteinase K and a special lysis buffer containing a chaotropic salt.
② Magnetic glass particles (MGPs) are added and nucleic acids are immobilized on the MGP surfaces.
③ Unbound substances such as proteins, cell debris, and PCR inhibitors are removed by several washing steps.
④ Purified nucleic acids are eluted from the MGPs.

5.2. Quality Control

The kit is function tested using several model systems:
- Quality of genomic DNA purified from human whole blood is tested using agarose gel electrophoresis and real-time PCR with the LightCycler® Carousel-Based Instrument targeting Cyclophilin A.
- Quality of total nucleic acid purified from human plasma spiked with Parvo Virus B19, Hepatitis A Virus (HAV), or Phocine Herpes Virus 1 (PhHV-1), is tested using real-time PCR with the LightCycler® Carousel-Based Instrument targeting Parvo Virus B19 or HAV and on the ABI PRISM Sequence Detection System 7700 targeting PhHV-1.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to Previous Version

- Layout changes
- Editorial changes

6.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage lifescience.roche.com.

Product	Pack size	Cat. No
Instruments		
MagNA Pure Compact Instrument	1 instrument with integrated PC, touchscreen monitor and barcode reader	03 731 146 001
Reagents , kits		
MagNA Pure Compact Nucleic Acid Isolation Kit I	1 kit 32 isolations	03 730 964 001
MagNA Pure Compact RNA Isolation Kit	1 kit 32 isolations	04 802 993 001
MagNA Pure DNA Tissue Lysis Buffer	100 ml lysis buffer	04 805 160 001
MagNA Pure Compact Tip Tray Kit	10 tip trays	03 753 166 001

6.4. Trademarks

HYBPROBE, LIGHTCYCLER, and MAGNA PURE are trademarks of Roche.
All other product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to: http://technical-support.roche.com.

6.6. Regulatory Disclaimer

For general laboratory use.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
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