For general laboratory use.

MagNA Pure LC DNA Isolation Kit I

Version 25

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Kit for isolation of genomic DNA from mammalian whole blood, blood cells or cultured cells, using MagNA Pure LC Instruments

Cat. No. 03 003 990 001 192 isolations

Store the kit at +15 to +25°C

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1. What this Product Does

Number of Tests

192 isolations (6 × 32) from up to

- 200 µl mammalian whole blood
- 1×10^6 blood cells or cultured cells

The kit is designed to process up to 192 samples in batches of 32. When processing fewer than 32 samples at a time, some reagent will be wasted and the remaining reagent will not be enough to process the number of samples listed above.

Kit Contents

The Lysis/Binding Buffer contains a blue ingredient required for clot detection during automated DNA isolation by the MagNA Pure LC Instruments.

The bottles of the Wash Buffer I and the MGPs have both black caps, although the color-coding of MagNA Pure LC Software and Positioning Frames is referring to a caramel cap for the MGPs.

Bottle/ Cap	Label	Contents / Function
1 black	Wash Buffer I	• 2 bottles, 100 ml each • for removal of PCR inhibitors
2 blue	Wash Buffer II	• 2 bottles, 100 ml each • for removal of salts, proteins etc.
3 green	Lysis/Binding Buffer	• 1 bottle, 100 ml • for cell lysis and binding of DNA
4 pink	Proteinase K	• 6 glass vials, lyophilizate • for digestion of proteins
5 black	Magnetic Glass Particles (MGPs) Suspension	• 6 vials, MGP suspension • for binding of DNA
6 yellow	Elution Buffer	• 1 bottle, 100 ml • for elution of DNA • for reconstitution of Proteinase K • for dilution of eluates (optional)

Storage and Stability

Kit components are stable at +15 to +25°C until the expiration date printed on the label.

Additional Equipment and Reagents Required

- standard laboratory equipment
- pipettes and nuclease-free, aerosol-preventive tips, to predispense samples into the MagNA Pure LC Sample Cartridge
- centrifuge and suitable nuclease-free reaction tubes
- vortex mixer, to resuspend the MGPs
- heating device (+65°C/+95°C, for reaction tubes: 1.5 to 2 ml)
- optionally required for some applications
  - PBS* (for pre-isolation of blood cells)
  - Red Blood Cell Lysis Buffer* (for pre-isolation of WBCs)
  - Vacutainer CPT tube (BD Diagnostics; for pre-isolation of PBMCs)
  - hemocytometer (*e.g.*, Neubauer device with counting chambers)

** available from Roche Life Science; see Ordering Information for details.*

Application

For general laboratory use. The MagNA Pure LC DNA Isolation Kit I is specially designed for use with MagNA Pure LC 2.0 Instrument (Cat. No. 05 197 686 001)], to isolate highly purified genomic DNA from mammalian whole blood or blood cells (WBCs, PBMCs), or cultured cells. The purified DNA can be used in PCR using LightCycler® Instruments or standard thermal block cyclers.

Assay Time

Set-up of the MagNA Pure LC Instrument requires approximately 15 min.
Total time for the automated purification of DNA from 32 samples is approximately:

- 45 to 90 min, for the "DNA I Blood_Cells High Performance" or "DNA I High_Performance_external_lysis" purification protocol
- 30 to 60 min, for the "DNA I Blood_Cells Fast" purification protocol

No hands-on time is required after set-up of the MagNA Pure LC Instruments. Extra hands-on time is required for the manual pre-isolation steps.

2. How to Use this Product

2.1 Before You Begin

Precautions

I) Handling Requirements

- Complete each phase of the PCR workflow before proceeding to the next phase. For example, you should finish PCR sample preparation before starting PCR set-up. Sample preparation, PCR set-up and the PCR run itself should also be performed in separate locations.
- Do not pool reagents from different lots or from different bottles of the same lot.
- Do not use a kit after its expiration date has passed.
- Wash Buffer I (bottle 1) and Lysis/Binding Buffer (bottle 4) contain guanidinium salts, which are irritants. Do not let Wash Buffer I or Lysis/Binding Buffer touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If you spill the reagent, dilute the spill with water before wiping it up.
- Do not allow the Lysis/Binding Buffer to mix with sodium hypochlorite (bleach) solution or strong acids. This mixture can produce a highly toxic gas.

II) 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.

III) Waste Handling

- Discard unused reagents and waste in accordance with country, federal, state and local regulations.
- Material Safety Data Sheets (MSDS) are available upon request from the local Roche office.

IV) For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

Purification Protocol

To process blood and cell samples with the MagNA Pure LC DNA Isolation Kit I, one of the three protocols listed below can be selected on the protocol selection menu of the 'Sample Ordering' screen of the MagNA Pure LC Software Version 3.0 or above. No extra protocol installation is required.

For MagNA Pure LC Software, Version 2.11 (or lower), these three blood- and cell-specific protocols must first be installed. For additional details, contact your local Roche representative.

Use the following information below to decide which purification protocol is best suited for your sample material.

Use the following table to determine which protocol is best for your sample material and application:

Protocol Name	Sample Material	Procedure
DNA I Blood_-Cells_High_Performance	• whole blood • blood cells • cultured cells	• This protocol is designed to achieve maximum DNA yield and PCR performance. It performs extended binding and elution steps, thereby slightly shearing the high molecular weight DNA for better amplification. • Sample volume: 20 to 200 µl • Elution volume: 100 µl
DNA I High_Performance_External_Lysis	• whole blood • blood cells • cultured cells	• This protocol is identical to the above “High Performance” protocol, with the exception that it enables processing of samples that have been lysed externally from the MagNA Pure LC Instrument. This is of advantage, if the site of sampling is separated from the site of DNA isolation. In this case, stability of DNA is ensured by adding Lysis/Binding Buffer to the sample, which inactivates nucleases. Also, use this protocol if samples, potentially containing pathogenic organisms, need to be inactivated in a Biosafety cabinet. • Sample volume: 20 to 200 µl • Elution volume: 100 µl

Protocol Name	Sample Material	Procedure
DNA I Blood_-Cells_Fast	• whole blood • blood cells • cultured cells	• This protocol is designed for applications such as mutation analysis, that do not require maximum DNA yield. It achieves about half the yield of the "High Performance" protocol. • Sample volume: 20 to 200 µl • Elution volume: 100 µl

⚠ All "DNA I" purification protocols enable the eluate to be diluted with up to 900 ml Elution Buffer.

Sample Material

To obtain optimal results in downstream procedures, especially in real-time PCR assays using LightCycler® Instruments, do not process samples with higher volume of cell count than this kit is designed to handle.
The optimal amount of sample material is, as follows:

- up to 200 µl whole blood (treated either with EDTA or citrate as anti-coagulant), containing maximally 1×10^6 WBCs or PBMCs
- up to 1×10^6 blood cells (WBCs or PBMCs), or cultured cells.

⚠ Do not use more sample material than this kit is designed to handle. Doing so may affect the performance of the isolation process and may lead to clumping and loss of the MGPs, or cross-contamination of samples.

⚠ Do not process whole blood samples containing more than 1×10^6 WBCs or PBMCs in a single sample. The actual concentration of WBCs and PBMCs in blood may differ from the values given above. If you are working at the upper limit of cell number (*i.e.,* 1×10^6 blood cells), always count the WBCs or PBMCs with a hemocytometer before using them in a sample and dilute the blood with PBS prior to use. Note that automatic counting systems (depending on the supplier) sometimes produce cell counts that do not agree with manual hemocytometer counts.

⚠ The "DNA I" purification protocols were developed with human whole blood. Remember that 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. Blood collected from different blood donors may contain different concentrations of blood cells. If you expect extremely high blood cell counts in a sample, use less (*e.g.,* 100 µl, instead of 200 µl) or dilute the sample (*e.g.* with PBS).

⚠ Treat all samples as potentially infectious.

⚠ It is best to use fresh or frozen samples. Avoid the use of samples that were stored at +15 to +25°C. Whole blood may be stored at +15 to +25°C for up to 1 day or at +2 to +8°C for up to 1 week. For longer storage times, whole blood samples should be frozen.

2.2 Preparation of Working Solutions

Before starting the procedure, prepare the working solutions as described below.

All other solutions are ready-to-use.

⚠ All buffers are clear. Do not use a buffer, if it contains a precipitate. If a precipitate is visible, place the bottle at +37°C and mix from time to time until the precipitate is completely dissolved. Do not warm the buffer longer at +37°C than is actually needed for complete dissolution of the precipitate. Before using the buffer, equilibrate at +15 to +25°C.

⚠ Equilibrate buffers and working solutions to +15 to +25°C before use. If you use the reagents at temperatures outside the recommended range, the kit may not function properly.

⚠ Use only the reagent amount required for your number of samples.

⚠ Do not store the Proteinase K or the MGP suspension in a Reagent Tub, or similar. All other reagents remaining in the Reagent Tubs after completion of the run, may be used for the next run, if performed on the same day. Longer storage periods are not recommended.

Reagent	Preparation/Comments	Storage
Magnetic Glass Particles	The MGP suspension (vial 5) must be mixed thoroughly. Vortex immediately before use to produce a homogeneous suspension. The beads tend to sediment during storage. ⚠ For best results, add the MGPs to the Instrument just before starting the run (to minimize sedimentation). Always use the exact amount of MGPs recommended by the software.	• Store MGPs at +15 to +25°C. ⚠ Do not store the MGP suspension in a Reagent Tub, or similar. ⚠ Do not leave the MGP suspension uncovered in the bottle or in the Reagent Tub, as evaporation may lead to suboptimal purification.

Reagent	Preparation/Comments	Storage
Proteinase K	Reconstitute each vial of Proteinase K (vial 4) by first adding 3.0 ml Elution Buffer (bottle 6). Close the vial and mix well, to completely dissolve the lyophilizate. After complete solubilization, add an additional 2.0 ml of the Elution Buffer to reach the final volume of 5.0 ml and mix again. After dissolving, the Proteinase K solution may appear turbid. This is caused by stabilizing components added to the Proteinase K. This appearance has no impact on the functionality of the enzyme. One bottle of Proteinase K is sufficient for 32 samples.	Once reconstituted, the Proteinase K is stable for 1 month at +2 to +8°C, or up to 12 months at −15 to −25°C.

Controls

Always run appropriate controls with the samples, especially if you want to perform quantification analyses of the eluted DNA samples (*e.g.,* by real-time PCR assays on the LightCycler® Instruments). 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 (*e.g.,* plasmid DNA) to all samples to be purified.

The IC is added prior to the purification step and then co-purified and amplified with your target of interest 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 may be reduced by inhibitors present in the purified sample material. In addition, the IC is used to compensate for possible losses of your target during purification.

For 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. 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® System, read the LightCycler® Technical Note 12/2000 “Absolute Quantification with External Standards and an Internal Control”, available at http://www.lightcycler-online.com.

2.3 Pre-Isolation Steps

Whole Blood

- No sample preparation is necessary: Transfer 20 to 200 µl whole blood directly into the Sample Cartridge.
- When using the "High_Performance_External_Lysis" protocol, add 300 µl Lysis/Binding Buffer to the blood sample and mix well. Either transfer the lysate directly to the Sample Cartridge or store for later use (for long-term storage, freeze at -15 to -25°C).

⚠ Adjust blood volumes < 200 µl to 200 µl with PBS.

⚠ If you assume that blood samples contain blood cell numbers greater than 1×10^6 cells, use less sample (dilute sample) to avoid overloading.

Blood samples can be stored at +15 to +25°C for at least 1 day, without any loss in DNA yield, or for up to 1 week with only a moderate loss in yield. For long-term storage, blood samples should be frozen.

WBCs

For manual pre-isolation of up to 1×10^6 WBCs from fresh, stabilized mammalian whole blood, follow the procedure below:

❶	Warm the Red Blood Cell Lysis Buffer* to + 15 to + 25 °C, and chill the PBS on ice. You will need two volumes of Red Blood Cell Lysis Buffer for every volume of blood processed.
❷	Add 1 part fresh blood to 2 parts Red Blood Cell Lysis Buffer.
❸	Incubate at + 15 to + 25 °C for 15 min on a roller incubator, until you see a clear solution (indicating complete red blood cell lysis). ⚠ **Do not vortex!**
❹	Centrifuge 10 min at 700 × *g* (at + 15 to + 25 °C).
❺	Carefully remove the supernatant with a pipet and discard.
❻	Suspend the WBC pellet in 1 ml Red Blood Cell Lysis Buffer and transfer into an Eppendorf vial.
❼	Centrifuge 3 min at 600 × *g* (at + 15 to + 25 °C).
❽	Carefully remove the supernatant with a pipet and discard.
❾	Suspend the white WBC pellet in cold PBS and store on ice, until you obtain a cell count.
❿	• Remove an aliquot from the WBC suspension, containing no more than 1×10^6 cells. • Centrifuge 3 min at 600 × *g* (at + 15 to + 25 °C).

⓫	• Carefully remove the supernatant with a pipet and discard. • Resuspend the cell pellet in 200 µl PBS. After the cells are suspended, perform the next step immediately. • (Optionally) When using the "High_Performance_External_Lysis" protocol, add 300 µl Lysis/Binding Buffer to the cell suspension and mix well. Either transfer the lysate directly to the Sample Cartridge or store for later use (for long-term storage, freeze at -15 to -25°C).
⓬	Transfer the suspension into the Sample Cartridge.
⓭	Place the Sample Cartridge on the Reagent/Sample Stage, and start the appropriate "DNA I" protocol, as described in section 2.4.

PBMCs

For manual pre-isolation of up to 1×10^6 PBMCs from fresh, stabilized mammalian whole blood, follow the procedure below:

❶	Collect up to 8 ml mammalian blood in a Vacutainer CPT tube (BD Diagnostics). Then isolate PBMCs from the blood, according to the instructions provided by the tube supplier.
❷	Transfer the supernatant, containing the PBMCs into a 15 ml Falcon tube and store on ice, until you obtain a cell count.
❸	• Remove an aliquot from the PBMC suspension, containing no more than 1×10^6 cells. • Add 1 part cold PBS to 1 part PBMCs suspension.
❹	Centrifuge 15 min at 300 × *g* (at +15 to +25°C).
❺	• Carefully remove the supernatant with a pipet and discard. • Resuspend the cell pellet in 200 µl PBS. Once the cells are suspended, perform the next step immediately. • (Optionally) When using the "High_Performance_External_Lysis" protocol, add 300 µl Lysis/Binding Buffer to the cell suspension and mix well. Either transfer the lysate directly to the Sample Cartridge or store for later use (for long-term storage, freeze at -15 to -25°C).
❻	Transfer the suspension into the Sample Cartridge.
❼	Place the Sample Cartridge on the Reagent/Sample Stage and start the appropriate "DNA I" protocol, as described in section 2.4.

Cultured Cells

For manual pre-isolation of up to 1×10^6 cultured cells, follow the procedure below:

❶	Pellet cells by centrifugation (10 min at 300 × *g*).
❷	• Carefully remove the supernatant with a pipet and discard. • Resuspend the cell pellet in 200 µl PBS. After the cells are suspended, perform the next step immediately. • (Optionally) When using the "High_Performance_External_Lysis" protocol, add 300 µl Lysis/Binding Buffer to the cell suspension and mix well. Either transfer the lysate directly to the Sample Cartridge or store for later use (for long-term storage, freeze at -15 to -25°C).
❸	Transfer the suspension into the Sample Cartridge.
❹	Place the Sample Cartridge on the Reagent/Sample Stage and start the appropriate "DNA I" protocol, as described in section 2.4.

2.4 DNA Isolation Protocol

General Remarks

- The following procedure is designed to process 32 samples at the same time. If you are processing fewer samples, the software will reduce the volumes of all solutions accordingly (see the 'Stage Setup' sub-tab of the MagNA Pure LC 2.0 Instrument).
- The software automatically calculates the necessary amounts of reagents and disposable plastics and guides you through the set-up.
- You can not start the Instrument unless the Disposable Lockbar, for securing the Sample Cartridge, Reagent Tubs and Reaction Tips, is closed.
- If you programmed dilution of the eluate, you will need an additional Reagent Tub M30 in position R7. Use Elution Buffer or nuclease-free 10 mM Tris-HCl, pH 8.0 as dilution buffer.

Protocol

Isolate genomic DNA according to the protocol below:

Start Instrument and Software	
MagNA Pure LC 2.0 Instrument	• Turn on the Instrument, the MagNA Pure LC 2.0 Software starts automatically. • Log in and then navigate to the 'Ordering' sub-tab.
	• Select the appropriate protocol: **If you are starting from.....** / **Then use....** • unlysed whole blood, blood cells, or cultured cell samples → • the "DNA I Blood_Cells_High_Performance" or "DNA I Blood_Cells_Fast" protocol. • externally lysed whole blood, blood cells, or cultured cell samples → • the "DNA I High Performance_External_Lysis" protocol.
	• Follow the instructions of the Software and specify the name and number of samples. Type in Sample Volume, Elution Volume and Dilution Volume (if necessary). The software will calculate how much of each reagent is required

Fill the Reagent Tubs	
	Before starting the isolation procedure, fill all Reagent Tubs outside the Instrument with the required amount of reagents (equilibrated to room temperature).
	Fill each Reagent Tub with the volume listed on the 'Stage Setup' sub-tab, then close it with a Tub Lid.
	⚠ Close Reagent Tubs with the Tub Lids, in order to prevent evaporation of the reagents. However, even when closed, Reagent Tubs are not suitable for long-term storage of reagents.
	⚠ Load the exact amount of MGPs (as listed on the 'Start Information' screen or 'Stage Setup' sub-tab) on to the Instrument, just before the run starts. This will prevent them from sedimenting.

Set Up Reagent Tubs and Disposables on the Reagent/Sample Stage

Use the information of the 'Stage Setup' sub-tab to place all disposable plastics and reagents within Reagent Tubs, necessary for the batch run on the Reagent/Sample Stage.

A colored "Positioning Frame"* that can be placed on the Reagent Reservoir Rack to aid correct loading of reagents is available with MagNA Pure LC Disposables Starter Set.

Load the Samples

- Transfer the Sample Cartridge, containing the samples or lysates to the MagNA Pure LC Instrument.
- Close the Disposable Lockbar.

Start the Batch Run

- On the 'Stage Setup' sub-tab, confirm the correct placement of all disposable plastics and reagents, by selecting the respective button on the Reagent/Sample Stage Area.
- Select the 'Start' button, to start the automated DNA isolation procedure. The Instrument will automatically dispense all reagents and process the samples.

Storage of DNA Eluates

⚠ To ensure greatest possible stability of the eluted nucleic acids, immediately proceed with PCR set up. Do not store the eluted nucleic acids in the MagNA Pure LC Storage Cartridge on Cooling Unit 1.

For storage, close the Storage Cartridge with a MagNA Pure LC Cartridge Seal* and store the DNA at −15 to −25°C (stable for at least several weeks). However, for long-term storage, it is recommended to store the DNA in aliquots in for e.g. screw-capped tubes at 15 to -25°C. so that the preparation will not be repeatedly frozen and thawed for later analyses.

⚠ After thawing eluates, mix gently by pipetting up and down ten times before performing any downstrean steps, *e.g.,* RT-PCR, or OD measurements. If nucleic acids are **not** premixed and distributed homogeneously in solution, results may not be reproducible in subsequent assays.

Post Elution Steps

The MagNA Pure LC Instrument can set up PCR reactions, by pipetting DNA samples and master mixes for PCR into either LightCycler® Capillaries*, standard PCR tubes or plates. (See the MagNA Pure LC Operator's Manuals for recommended plates.) For Post Elution procedures, you can place LightCycler® Capillaries in the removable MagNA Pure LC Cooling Block, LC Centrifuge Adapters*, or the MagNA Pure LC Cooling Block, LC Sample Carousel*. Alternatively, you can place a LightCycler® 480 Multiwell Plate 96* into the MagNA Pure LC Cooling Block, 96-well PCR Plate*, in combination with the MagNA Pure LC LightCycler® 480 Plate Adapter*. You can program the Post Elution steps either before you perform the isolation procedure, or after it is complete. See the MagNA Pure LC Operator's Manuals, for details on how to set up a Post Elution run.

3. Results

Integrity

Figure 1: DNA was purified from whole blood, K562 and HeLa cells using the MagNA Pure LC DNA Isolation Kit I and a major competitor's manual DNA isolation method. Isolated DNA was analyzed by agarose gel electrophoresis. DNA was of high quality, with an average molecular weight of approx. 50 kb and showed no signs of degradation (see Fig. 1). DNA isolated using the MagNA Pure LC Instrument was of higher to integrity and purity (less RNA contamination).

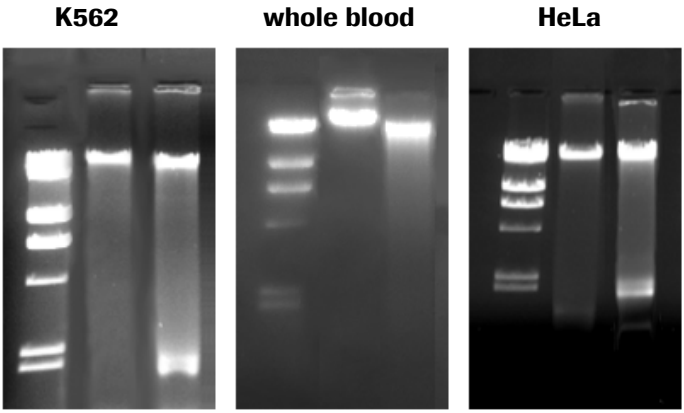


Fig. 2: Lane 1: Molecular Weight Marker XV*; Lane 2: DNA isolated using the MagNA Pure LC Instrument; Lane 3: DNA isolated using a competitor's manual method.

Yield and Purity

DNA was prepared from different types of cultured cells and from human whole blood. The scalability of yield was determined using different amounts of starting material. Yields were determined by OD_{260} measurement and agarose gel analysis. Compared to conventional DNA isolation methods using filter tube-based methods), the MagNA Pure LC Isolation Method results in equal or higher DNA yields (see table below).

Typical experiments yielded DNA with an $OD_{260/280}$ ratio of 1.9 ± 0.1, indicating high purity. No inhibition in real-time PCR assays was observed using the LightCycler® Carousel-Based System (see table below).

Sample type	DNA yield [µg]	Purity $OD_{260/280}$	Crossing Point [1]
Whole Blood			
20 µl	1.5	1.9 ± 0.1	25.6
50 µl	3.2		24.8
100 µl	4.6		24.7
200 µl	7.2		23.6
Cultured cells (K562)			
1×10^3	- [2]	1.9 ± 0.1	34.5
1×10^4	- [2]		30.1
1×10^5	1.8		25.8
2.5×10^5	3.5		24.3
5×10^5	6.0		23.4
1×10^6	10.6		23.1
Cultured cells (HeLa)			
1×10^3	- [2]	1.9 ± 0.1	35.8
1×10^4	- [2]		30.2
1×10^5	2.1		25.8
2.5×10^5	5.7		24.3
5×10^5	6.0		24.4
1×10^6	11.1		23.8

[1] The crossing point (Cp) value reflects the isolated amount of DNA and is calculated after real-time PCR analysis (Factor V Leiden) using the LightCycler® System. Quantification is based on a DNA standard.

[2] OD_{260} not measurable due to very low amounts of DNA isolated from given amounts of sample.

Removal of Anticoagulants

Blood samples containing different anticoagulants (EDTA, citrate) were subjected to the "DNA I Blood_Cells_High_Performance" protocol. No PCR inhibition was observed in real-time PCR assays on the LightCycler® Carousel-Based System.

Reproducibility

- **Intra-assay variance:** Thirty-two samples of 20 µl whole blood or of 10^5 K562 cells were processed according to the "DNA I" purification protocols. The coefficient of variance (CV) of DNA yield and purity was <10% and the CV for the crossing points was 3%.
- **Inter-assay variance:** Six samples of 20 µl whole blood were subjected to the "DNA I Blood_Cells_High_Performance" or the "DNA I Blood_Cells_Fast" protocol in 5 independent runs. The CV of DNA yield and purity was < 10% and the CV for the crossing points was < 3%.

Downstream Applications

DNA from different types of sample materials (whole blood, blood cells, cultured cells) was tested by real-time PCR using the LightCycler® Carousel-Based System, by PCR on conventional block cyclers and restriction digests and Southern blotting. Purified DNA was suitable for all types of experiments and showed identical or improved performance compared to DNA prepared by conventional isolation methods.

4. Troubleshooting

	Possible Cause	Recommendation
Clumping of beads or presence of beads in Storage Cartridge	Too much sample material	Reduce amount of sample material to the values indicated in the section “Sample Material”.
	MGPs were magnetized prior to use.	• Avoid contact between the MGPs and magnets prior to use. • Store the kit appropriately.
DNA is degraded.	Storage of samples was not appropriate.	• Use fresh or frozen samples, whenever possible. • Avoid the use of samples that have been stored extensively at room temperature (+15 to +25°C).
Poor DNA yield	Sample does not contain enough cells.	**Cells:** count cells from cell culture before use. Optimal results are obtained with 10^5 cells (possible range 10^2 to 10^6 cells). **Blood:** ensure that the blood is not clotted and that the blood cells are not sedimented. Use fresh or frozen anticoagulated blood. Shake or vortex tubes before use.
	Storage of samples was not optimal.	• Use fresh or frozen samples, whenever possible. • Avoid the use of samples that have been stored extensively at room temperature (+15 to +25°C).
Poor DNA purity	Too much sample material	Reduce amount of sample material to the values indicated in the section “Sample Material”.
Poor PCR performance	Too much DNA in PCR	• Check DNA concentration of eluates and adjust amount of eluate per PCR or dilute the eluate (*e.g.*, 1 in 10). Optimum: 1 to 100 ng/PCR, maximum: approx. 1 µg. • Use less starting material. Optimal results are obtained with 20 µl whole blood or 10^5 cells.
	Not enough DNA in PCR	• Check DNA concentration of eluates and adjust amount of eluate per PCR or dilute the eluate (*e.g.*, 1 in 10). Optimum: 1 to 100 ng/PCR maximum: approx. 1 µg • Use less starting material. Optimal results are obtained with 20 µl whole blood or 10^5 cells
	Poor purity of DNA	Use less sample material for DNA isolation. Optimal results are obtained with 20 µl whole blood or 10^5 cells.
	PCR reagents and protocols were not optimal.	Check PCR reagents and protocols with a positive DNA control (*e.g.,* human genomic DNA*).
	Denaturation too short	Optimize PCR, *e.g.,* include an initial denaturation step of 30 s to 3 min in the PCR protocol.
	High Heparin concentration in blood sample	Use the “DNA I Blood_Cells_Fast” protocol, instead of the “DNA I Blood_Cells High Performance” protocol, or use EDTA- or citrate-stabilized blood.
Eluates show a slight 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 PCR using LightCycler® Instruments.

5. Additional Information on this Product

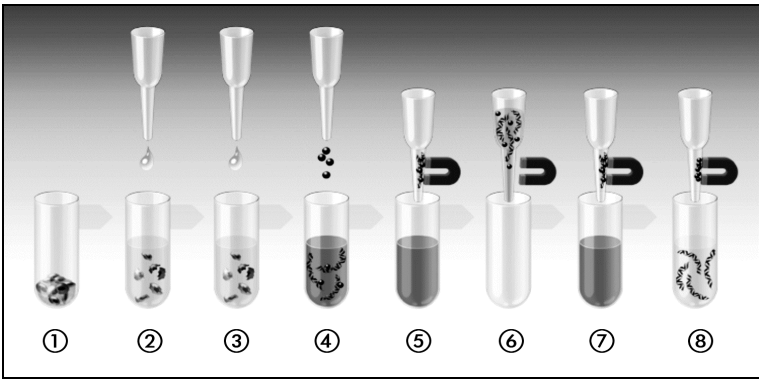
5.1 How this Product Works

The MagNA Pure LC DNA Kit I is used with the MagNA Pure LC Instrument to purify high-quality, undegraded genomic DNA from 1 to 32 samples of mammalian whole blood, blood cells, or cultured cells. Isolated DNA meets the quality standards required for highly sensitive and quantitative PCR analysis using LightCycler® Instruments.

Test Principle

The isolation procedure uses magnetic-bead technology. The samples are lysed by incubation using a special buffer containing a chaotropic salt and Proteinase K. Magnetic Glass Particles (MGPs) are added and DNA is bound to their surfaces. Unbound substances are removed by several washing steps, and purified DNA is eluted using a low salt buffer.

The principle steps of a MagNA Pure LC DNA isolation procedure are:



①	Sample material is placed into the wells of the Sample Cartridge.
②	Lysis/Binding Buffer is added to the sample, resulting in complete cell lysis and release of nucleic acids. Nucleases are denatured.
③	Proteinase K is added to the samples and proteins are digested.
④	DNA binds to the silica surface of the added MGPs, due to the chaotropic salt conditions, isopropanol and the high ionic strength of the Lysis/Binding Buffer.
⑤	MGPs with bound DNA are magnetically separated from the residual lysed sample.

⑥	MGPs with bound DNA are washed repeatedly with Wash Buffer to remove unbound substances [*e.g.* proteins (nucleases), cell membranes and PCR inhibitors, such as heparin or hemoglobin], and to reduce the chaotropic salt concentration.
⑦	MGPs with bound DNA are magnetically separated from the Wash Buffer containing residual sample debris.
⑧	Purified DNA is eluted from the MGPs in the wells of the Elution Cartridge. MGPs are retained in the Reaction Tip and discarded.

The basic steps of the MagNA Pure LC DNA Isolation Kit I isolation procedure ("DNA I Blood_Cells_High_Performance" protocol) are as follows:

DNA Isolation is performed using automation by MagNA Pure LC Instrument	
①	Dispense all required reagents into the Processing Cartridge.
②	Dispense Elution Buffer into the Elution Cartridge (Heating Unit).
③	Add Lysis/Binding Buffer to the sample, then mix.
④	Transfer lysate into Proteinase K solution, then mix.
⑤	Transfer lysate into MGP suspension, then mix and incubate.
⑥	Transfer MGPs into Wash Buffer I, mix, separate particles.
⑦	Transfer MGPs into Wash Buffer II, then mix, separate particles.
⑧	Transfer MGPs into Wash Buffer II again, then mix, separate particles.
⑨	Transfer MGPs into the Elution Buffer, mix, incubate, elute DNA.
⑩	Shear DNA by pipetting 5 min using small Reaction Tips.
⑪	Separate and discard MGPs.
⑫	Transfer eluate to the Storage Cartridge (Cooling Unit I).

5.2 Quality Control

DNA is isolated from human whole blood and cultured cells using the protocol "DNA I Blood_Cells_High_Performance" and analyzed with respect to integrity, yield, purity and ability to amplify in a real-time PCR assay using the LightCycler® System. For typical experimental data, please refer to section 3.

5.3 References

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5.4 Product Citations

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6. Supplementary Information

6.1 Conventions

Text Conventions To make information consistent and easy to understand, the following text conventions are used in this document:

Text Convention	Usage
Numbered stages labeled ①, ② etc.	Stages in a process that usually occur in the order listed.
Numbered instructions labeled ❶, ❷ etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Diagnostics.

Symbols In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

Abbreviations In this document, the following abbreviations are used:

Abbreviation	Meaning
Cp	crossing point
CV	coefficient of variance
MGP	magnetic glass particle
PBMC	peripheral blood mononuclear cell
WBC	white blood cells

6.2 Changes to Previous Version

- New information added related to the REACH Annex XIV.

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, www.lifescience.roche.com, and our Special Interest Sites including

- Automated Sample Preparation (MagNA Lyser Instrument, MagNA Pure Compact System and MagNA Pure LC Systems): www.magnapure.com
- Real-time PCR Systems (LightCycler® System, LightCycler® 480 System and Universal ProbeLibrary): www.lightcycler.com

Instruments and Accessories

Product	Pack Size	Cat. No.
MagNA Pure LC 2.0 Instrument	1 instrument plus accessories	05 197 686 001
MagNA Pure LC Cooling Block, LC Centrifuge Adapters	1 cooling block with 32 LightCycler® Centrifuge Adapters	12 190 664 001
MagNA Pure LC Cooling Block, LC Sample Carousel	1 cooling block	12 189 704 001
MagNA Pure LC Cooling Block, 96-well PCR Plate	1 cooling block	12 189 674 001
MagNA Pure LC LightCycler® 480 Plate Adapter	1 adapter	05 323 983 001
MagNA Pure LC Cartridge Seals	200 seals	03 118 827 001
Positioning Frame	only available with the MagNA Pure LC Disposables Starter Set	03 005 488 001
LightCycler® 480 Instrument II	1 instrument (96 well) 1 instrument (384 well)	05 015 278 001 05 015 243 001
LightCycler® 480 Multiwell Plate 96, white	5 × 10 plates (incl. sealing foil)	04 729 692 001
LightCycler® 480 Multiwell Plate 96, clear	5 × 10 plates (incl. sealing foil)	05 102 413 001
LightCycler® 2.0 Instrument	1 instrument plus accessories	03 531 414 001
LightCycler® 1.5 Instrument	1 instrument plus accessories	04 484 495 001

	Product	Pack Size	Cat. No.
	LightCycler® Capillaries (20 µl)	1 pack (5 boxes, each with 96 capillaries and stoppers)	04 929 292 001
	LC Carousel Centrifuge 2.0	1 centrifuge plus rotor (230 V) 1 centrifuge plus rotor (115 V)	03 709 582 001 03 709 507 001
Kits for DNA Isolation	MagNA Pure LC DNA Isolation Kit II (Tissue)[1)]	1 kit (192 isolations)	03 186 229 001
	MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi)[1)]	1 kit (192 isolations)	03 264 785 001
	MagNA Pure LC DNA Isolation Kit - Large Volume[1)]	1 kit (96 to 288 isolations)	03 310 515 001
Kits for RNA Isolation	MagNA Pure LC RNA Isolation Kit – High Performance[1)]	1 kit (192 isolations)	03 542 394 001
	MagNA Pure LC RNA Isolation Kit III (Tissue)[1)]	1 kit (192 isolations)	03 330 591 001
Kits for Total Nucleic Acid Isolation	MagNA Pure LC Total Nucleic Acid Isolation Kit[1)]	1 kit (192 isolations)	03 038 505 001
	MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume[1)]	1 kit (192 isolations)	03 264 793 001
	MagNA Pure LC Total Nucleic Acid Isolation Kit - High Performance[1)]	1 kit (96 to 288 isolations)	05 323 738 001
Associated Reagents	Buffers in a Box, Pre-mixed PBS Buffer, 10×	4 l	11 666 789 001
	Red Blood Cell Lysis Buffer	100 ml	11 814 389 001
	Human Genomic DNA	100 µg	11 691 112 001
	DNA Molecular Weight Marker XV	50 µg (1 A_{260} unit)	11 721 615 001

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