For general laboratory use.

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MagNA Pure LC Total Nucleic Acid Isolation Kit – Large Volume

Version 17

Content version: September 2015

Kit for the isolation of total nucleic acids from 1 ml of mammalian serum or plasma using the MagNA Pure LC Instrument

Cat. No. 03 264 793 001 Kit for 192 isolations

Store the kit at +15 to +25°C

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PROTOCOLS

1. What this Product Does

Number of Tests

192 isolations (6 × 32) from up to 1 ml of mammalian serum or plasma.

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

Kit Contents

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

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 removing PCR inhibitors
2 blue	Wash Buffer II	• 1 bottle, 100 ml • for removing salts, proteins etc.
3 red	Wash Buffer III	• 2 bottles, 100 ml each • for removing salt, proteins etc.
4 green	Lysis/Binding Buffer	• 4 bottles, 100 ml each • for cell lysis and binding of DNA
5 pink	Proteinase K	• 6 glass vials with lyophilizate • for digesting proteins
6 black	Magnetic Glass Particles (MGPs) Suspension	• 6 vials, 11 ml each • for binding of total nucleic acids
7 yellow	Elution Buffer	• 1 bottle, 100 ml • for elution of pure total nucleic acids • for reconstitution of Proteinase K • for dilution of eluates (optional)

Storage and Stability

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

⚠ Never store the MGPs in a Reagent Tub or similar.

Additional Equipment and Reagents Required

- standard laboratory equipment
- pipettes and nuclease-free, aerosol-preventive tips to predispense samples into the Sample Cartridge
- centrifuge and suitable nuclease-free reaction tubes
- vortex mixer

Application

The MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume, a General Purpose Reagent (GPR), is specifically designed for the purification of total nucleic acids from large amounts (up to 1 ml) of serum and plasma on the MagNA Pure LC Instrument. The purified total nucleic acid is suitable for highly sensitive and quantitative PCR and RT-PCR on the LightCycler® Instruments as well as for PCR and RT-PCR on standard thermal block cyclers.

Assay Time

Setup of the MagNA Pure LC Instrument requires approx. 15 min total time for the automated purification of DNA is approximately 30 – 120 min for 1 - 32 samples.

No hands-on time is required after setup of the MagNA Pure LC Instrument. 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 setup. Sample prep, PCR setup 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. 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 has to optimize pathogen inactivation by the Lysis/Binding Buffer or take appropriate measures according to local safety regulations. Roche does not warrant that samples treated with Lysis/Binding Buffer are completely inactivated and non-infectious. If you worked with potentially infectious sample material, remove and autoclave all disposable plastics (including the Liquid Waste Bottle with liquid waste and the Waste Bag with discarded Reaction Tips) after sample processing is completed.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coates and eye protection when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, viruses, 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 test 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.

Purification Protocol

To process the samples with the MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume, a new protocol for the MagNA Pure LC Software, Version 2.11 (or lower) must be installed. The name **"Total NA LV Serum_Plasma"** should appear in the protocol selection of the "Sample Ordering" screen of the MagNA Pure LC Software. If running software version 3.0 or above, no extra protocol installation is required. For additional details, contact your local Roche representative.

One protocol is available for the MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume:

Protocol Name	Procedure
Total NA LV Serum_Plasma	• Fully automated • Sample volume: 1,000 µl • Elution volume: 50 to 100 µl

Sample Material

The MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume has been designed for fully automated processing of 1,000 µl of mammalian serum or plasma. It is best to use fresh or frozen samples.

- You can process smaller sample volumes (500 to 1,000 µl) if you raise the volume of the sample to 1,000 µl by adding an appropriate buffer such as Elution buffer (vial 7, yellow cap).
- For isolation of total nucleic acids from small sample volumes (50 to 200 µl) use the MagNA Pure LC Total Nucleic Acid Isolation Kit*.
- Never use more sample material than this kit is designed to handle. Doing so may lead to loss of MGPs and affect the performance of the isolation process and of downstream analytical procedures.
- The MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume must not be used for the processing of whole blood samples.
- Treat all samples as potentially infectious.

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 it, bring the buffer back to +15 to +25°C.
- Incubate buffers at +15 to +25°C before use. If you use the reagents at temperature outside the recommended range, the kit may not work very well.
- Use only the reagent amount needed for your sample number.

⚠ Never store the Proteinase K and the MGP suspension in Reagent Tubs. All other reagents remaining in the Reagent Tubs after completion of the run can 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 6) 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. ⚠ **Never 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 of alcohol might lead to suboptimal purification.
Proteinase K	Reconstitute each bottle Proteinase K (vial 5) by adding 6.7 ml Elution Buffer (vial 7). Close the vial and mix well to completely dissolve the lyophilizate. ⚠ After dissolving, the Proteinase K solution might appear turbid. This is caused by stabilizing components added to Proteinase K. This appearance has no impact on functionality of the enzyme. One bottle 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 nucleic acid 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 (IC), by using a sample material positive for your target.
- Negative Control, by using a sample material negative for your target.
- Internal Control, by adding a defined amount of a control template 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 from the specimen in the same PCR or RT-PCR reaction. The IC concept is especially useful for enzyme-based amplification processes such as PCR or RT-PCR, because efficiency of the PCR or RT-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 quantification assays on the LightCycler® Instruments, use a synthetic double-stranded DNA molecule or *in vitro* transcribed RNA 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® Carousel-based System, read LightCycler® Technical Note 12/2000 "Absolute Quantification with External Standards and an Internal Control" available at http://www.lightcycler.com.

2.2 Procedure

2.2.1 Pre-Isolation Steps

The isolation of total nucleic acids from serum and plasma samples proceeds fully automated. No pre-isolation steps are required.

2.2.2 Total Nucleic Acid Isolation Protocol

General Remarks

- The following procedure is designed to process 32 samples at the same time. If you are processing fewer samples, reduce the volumes of all solutions accordingly (see the Start Information Screen of the MagNA Pure LC Instrument).
- The software automatically calculates the necessary amounts of reagents and guides you through the setup.
- You cannot start the instrument unless the interlock for securing Sample Cartridge, Reagent Tubs, and Reaction Tips is closed.

Protocol Isolate total nucleic acid according to the protocol.

Start Instrument and Software	• Turn on the instrument and the computer, then start the MagNA Pure LC software. Select the **Total NA LV Serum_Plasma** purification protocol.
	Follow the instructions of the software and specify the number of samples. Type in Sample Volume and Elution Volume. 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 (warmed to +15 to +25°C). Fill each Reagent Tub with the volume listed on the Start Information Screen, then cap 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) onto the instrument just before the run starts. This will keep them from sedimenting.
Set Up Reagent Tubs on Reagent/ Sample Stage	Use the information of the Start Information Screen 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 Tub Rack to aid correct loading of the reagents is available with the MagNA Pure LC Disposables Starter Set.
Load the Samples	Transfer the Sample Cartridge containing the sample to the MagNA Pure LC Instrument.
Start the Batch Run	• On the Start Information Screen, confirm the correct placement of all disposable plastics and reagents by mouse-clicking the respective text boxes. • Click the ‘OK’ button to start the automated RNA isolation procedure. The instrument will automatically dispense all reagents and process the samples.

Storage of total nucleic acid Eluates

⚠ To ensure greatest possible stability of the eluted nucleic acids, immediately proceed with PCR or RT-PCR setup. Do not store the eluted nucleic acid in the Storage Cartridge on Cooling Unit 1.

For long-term storage (at least for several weeks), close the Storage Cartridge with the Cartridge Seals* and store the nucleic acids at –15 to –25°C. It is best to store the nucleic acids in aliquots, so the preparation will not have to be repeatedly frozen and thawed.

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

Post-Elution Steps

The MagNA Pure LC Instrument can help set up PCR and RT-PCR reactions by pipetting nucleic acid samples and master reagent mixes for PCR or RT-PCR into either LightCycler® Capillaries, standard PCR tubes or plates. (See the MagNA Pure LC Operator's Manual for recommended plates.) For post-elution procedures, you can place LightCycler® Capillaries in the removable MagNA Pure LC Cooling Block, LC Centrifuge Adaptors or the MagNA Pure LC Cooling Block, LC Sample Carousel. 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 Manual for details how to set up a post-elution run.

3. Results

Scalability

Human Parvovirus B19 was serially diluted tenfold (replicates of 2) to the indicated viral concentrations and purified using the MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume (sample volume: 1,000 µl) and the MagNA Pure LC Total Nucleic Acid Isolation Kit (sample volume: 200 µl). Analysis was done using the LightCycler® Parvovirus B19 Quantification Kit on the LightCycler® 1.5 Instrument.

The mean difference in the crossing points for the corresponding samples was $\Delta Cp = 2.4$ as expected for a fivefold difference in the initial sample volume.

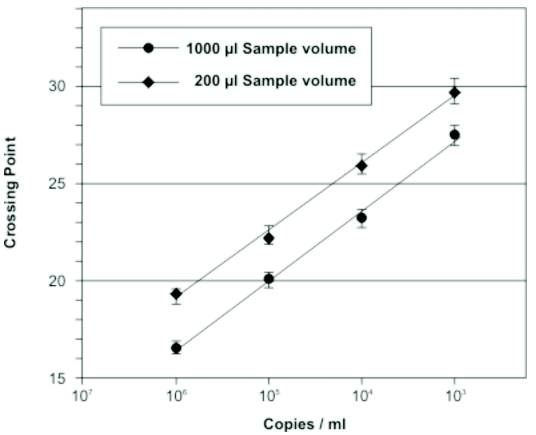


Fig. 1: LightCycler® System analysis of Parvovirus B19-positive, citrate plasma samples, after purification with the MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume (-●-) and the MagNA Pure LC Total Nucleic Acid Isolation Kit (-◆-).

Prevention of Cross Contamination

In general, sample materials with high virus titers require a higher amount of precaution during the handling procedure than others. Aerosol formation can play an important role during all processing steps involved. Aerosol formation may lead to sample contaminations which can be detected by low signals in negative control samples usually found in the range of > 35 Cps at a low frequency in real-time PCRs. These contaminations may occur during sample set up, the nucleic acid purification itself, PCR set up or the post PCR processing (laboratory contamination with amplicons).

In order to avoid any negative impact on analytical results, the following recommendations should strictly be followed:

- Define and establish for real-time PCR applications the lower limit of detection < 35 Cp (for example Cp 30-34). For conventional Heat Block Cycler applications implement similar methods (*e.g.* gel analysis).
- Whenever possible use UNG (Uracil-DNA Glycosylase, heat-labile, Cat No.: 11 775 367 001) to prevent carryover of PCR Amplicons from previous PCRs.
- Always confirm low positive results through an independent experiment.

Reproducibility (intra-assay variance)

30 replicates of a plasma sample positive for viral RNA were purified using the MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume, and the eluted nucleic acids were analyzed using the LightCycler® 1.5 Instrument. The coefficient of variance (CV %) calculated for the corresponding crossing points was < 2 %.

Reproducibility (inter-assay variance)

6 plasma samples positive for viral RNA were subjected to the standard total nucleic acid isolation protocol in 5 independent runs. Eluates were analyzed by the LightCycler® 1.5 Instrument. The coefficient of variance (CV %) calculated for corresponding crossing points was < 2 %.

	Possible Cause	Recommendation
Clumping of beads	Too much sample material.	Reduce amount of sample material to the values recommended in the section “sample material”.
	MGPs were magnetized prior to use.	Avoid contact of the MGPs with magnets prior to use.
Nucleic acid is degraded	Storage of samples was not appropriate.	Use fresh or frozen samples, avoid the use of samples that were extensively stored at +15 to +25°C.
Unclear UV spectrum	The Elution Buffer used in the MagNA Pure L C Total Nucleic Acid Isolation Kit Large Volume contains stabilizating components that interfere with standard OD_{260} measurements.	
Unexpected amount of eluate	Wrong elution volume has been set	Confirm correct setting of elution volume as indicated in the package insert.
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 negatively effect PCR assays on the LightCycler® Instruments.

5. Additional Information on this Product

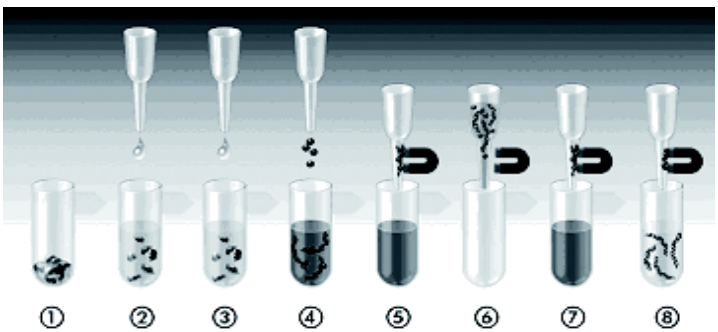
How this Product Works

MagNA Pure LC Total Nucleic Acid Kit – Large Volume is used with the MagNA Pure LC Instrument to purify high-quality, undegraded nucleic acids from 1 – 32 samples of mammalian serum and plasma samples. The isolated nucleic acid meets the quality standards required for highly sensitive and quantitative PCR analysis on the LightCycler® Instruments.

Test Principle

The isolation procedure is based on magnetic-bead technology. The samples are lysed by incubation with a special buffer that contains chemotropic salts and Proteinase K. Magnetic Glass Particles are added and the nucleic acid is bound to their surfaces. Unbound substances are removed by several washing steps, and then the purified nucleic acid is eluted.

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



①	The 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	Nucleic acid 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 nucleic acid are magnetically separated from the residual lysed sample.
⑥	MGPs with bound nucleic acid are washed repeatedly with Wash Buffer to remove unbound substances like proteins (nucleases), cell membranes, PCR inhibitors such as heparin or hemoglobin, and to reduce the chaotropic salt concentration.
⑦	Again MGPs with bound nucleic acid are magnetically separated from the Wash Buffer containing residual sample debris.

⑧	The purified nucleic acid is eluted at +70°C from the MGPs in the wells of the Elution Cartridge, whereas the MGPs are retained in the reaction tip and discarded.

The basic steps of the MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume procedure for serum and plasma samples are as follows:

Nucleic Acid Isolation Step	Performed automatically by the MagNA Pure LC Instrument
①	Dispense 100 µl MGPs into the 1st, 2nd, and 3rd row of the Processing Cartridge
②	Dispense all required Wash Buffers into the Processing Cartridge
③	Dispense 450 µl Lysis/Binding Buffer into the 1st, 2nd, and 3rd row of the Processing Cartridge.
④	Dispense 50 – 100 µl Elution Buffer into the Elution Cartridge (Heating Block)
⑤	Dispense 150 µl Proteinase K to samples in the Sample Cartridge, mix and incubate for 5 min.
⑥	Aspirate sample from the Sample Cartridge and dispense aliquots into the1st, 2nd, and 3rd row; then mix samples with the Lysis/Binding Buffer and MGPs.
⑦	Separate beads in the1st and 2nd. Move to 3rd row of Processing Cartridge, mix and separate.
⑧	Transfer beads into 850 µl Wash Buffer I in row 4 mix, and separate.
⑨	Transfer beads into 450 µl Wash Buffer II in row 5 mix, separate.
⑩	Transfer beads into 450 µl Wash Buffer III in row 6,7 mix, separate.
⑪	Transfer beads into Elution Buffer (Heating Unit), mix, incubate, and elute nucleic acids. Discard MGPs.
⑫	Transfer eluate to the Storage Cartridge (Cooling Unit I).

Quality Control

The Kit is function-tested by isolation of total viral nucleic acids (DNA and RNA) from Hepatitis A-positive and Parvo Virus B19-positive human reference material using the "Total NA LV Serum_Plasma" purification protocol. Purified total viral nucleic acids are then detected by quantitative real-time PCR and RT-PCR using virus specific assays established for the LightCycler® System.

Kit components are tested for the absence of nucleases, according to the current quality control procedures.

Reference

Dijkstra-Tiekstra, M.J. *et al.* (2004). Development of white blood cell fragments, during the preparation and storage of platelet concentrates, as measured by using real-time polymerase chain reaction. *Vox Sanguinis,* **87:** 250-256.

6. Supplementary Information

6.1 Conventions

Text Conventions To make information consistent and memorable, 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
HAV	Hepatitis A Virus

6.2 Changes to Previous Version

- 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 home page, www.lifescience.roche.com, and our Special Interest Sites including:

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

	Product	Pack Size	Cat. No.
Instruments and Accessories	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 Cartridge Seal	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	1 instrument (96 well) 1 instrument (384 well)	05 015 243 001 05 015 278 001
	LightCycler® 2.0 Instrument	1 instrument plus accessories	03 531 414 001
	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
MagNA Pure LC Kits for DNA Isolation	MagNA Pure LC DNA Isolation Kit II	1 kit (192 isolations)	03 186 229 001
	MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi)	1 kit (192 isolations)	03 264 785 001

	Product	Pack Size	Cat. No.
	MagNA Pure LC DNA Isolation Kit - Large Volume	1 Kit 96 isolations from 1 ml blood 192 isolations from 300 – 500 µl blood 288 isolations from 20 –200 µl blood 192 isolations from blood cells 192 isolations from 5×10^6 cultured cells	03 310 515 001
	MagNA Pure LC DNA Isolation Kit I – Lysis/binding Buffer Refill	70 ml	03 246 752 001
MagNA Pure LC Kits for RNA/ mRNA Isolation	MagNA Pure LC RNA Isolation Kit – High Performance	1 kit (192 isolations)	03 542 394 001
	MagNA Pure LC RNA Isolation Kit III (Tissue)	1 kit (192 isolations)	03 330 591 001
MagNA Pure LC Kits for Total Nucleic Acid Isolation	MagNA Pure LC Total Nucleic Acid Isolation Kit	1 kit (192 isolations)	03 038 505 001
	MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume	1 kit (192 reactions)	03 264 793 001
	MagNA Pure LC Total Nucleic Acid Isolation Kit I – Lysis/binding Buffer Refill	100 ml	03 246 779 001
Associated Kits and Reagents	RNase A	100 mg	10 109 169 001
	Human Genomic DNA	100 mg	11 691 112 001

6.4 Trademarks

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- Information Material

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