For general laboratory use.

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MagNA Pure LC Total Nucleic Acid Isolation Kit

Version 14

Content version: August 2014

Kit for isolation of total viral nucleic acids from mammalian serum, plasma and whole blood, using MagNA Pure LC Instruments

Cat. No. 03 038 505 001

192 isolations

Store the kit at +15 to +25°C

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1. What this Product Does

Number of Tests

- 192 isolations from up to 200 µl mammalian serum or plasma, or up to 100 µl mammalian whole blood.

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 192 samples.

Kit Contents

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

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	• 1 bottle, 100 ml • for removal of salts, proteins etc.
3 red	Wash Buffer III	• 2 bottles, 100 ml each • for removal of salts etc.
4 green	Lysis/Binding Buffer	• 1 bottle, 100 ml • for cell lysis and binding of total nucleic acid
5 pink	Proteinase K	• 6 glass vials, lyophilizate • for digestion of proteins
6 caramel	Magnetic Glass Particles (MGPs) Suspension	• 6 vials, 6 ml MGP suspension each • for binding of total nucleic acid
7 yellow	Elution Buffer	• 1 bottle, 100 ml • for elution of pure total nucleic acid • for dilution of eluates (optional) • for reconstitution of Proteinase K

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

Application

The MagNA Pure LC Total Nucleic Acid Isolation Kit, a General Purpose Reagent (GPR), is specially designed for use with the MagNA Pure LC Instruments [MagNA Pure LC 1.0 Instrument and MagNA Pure LC 2.0 Instrument (Cat. No. 05 197 686 001)], to isolate highly purified total viral nucleic acids (DNA and RNA) from mammalian serum, plasma, or whole blood. Purified total nucleic acids can be used both for PCR or RT-PCR using LightCycler® Instruments and standard thermal block cyclers. Purified total nucleic acids are free of PCR inhibitors.

Assay Time

Set-up of the MagNA Pure LC Instruments requires approximately 15 min. Total time for the automated purification of total nucleic acid from 32 samples is approximately 90 min.

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/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.
- 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.
- Regarding precautions for safe handling of RNA, see the LAB FAQS.
- 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.

Purification Protocol

To perform total viral nucleic acid isolations (DNA and RNA) on all MagNA Pure LC Instruments with the MagNA Pure LC Total Nucleic Acid Isolation Kit three different purification protocols are available. The protocol names, listed below, should appear in the protocol selection of the 'Sample Ordering' screen of the MagNA Pure LC 1.0 Instrument, or on the 'Ordering' sub-tab of the MagNA Pure LC 2.0 Instrument. Use the following table to determine which protocol is best for your sample material and application:

Protocol Name	Sample Material	Procedure
Total NA Serum_Plasma_ Blood	50 to 200 µl serum or plasma 50 to 100 µl whole blood	• fully automated • Sample volume: 50 to 200 µl • Elution volume: 100 µl
Total NA Variable_elution_ volume		• fully automated • Sample volume: 50 to 200 µl • Elution volume: 50 to 100 µl [1)]
Total NA External_lysis		• Samples are lysed manually, outside the MagNA Pure LC Instruments. Lysates are then transferred to the Reagent/ Sample Stage and purification is performed automatically by the Instrument. • Enables the physical separation of the lysis step from the purification step and to load inactivated sample material into the MagNA Pure LC Instruments (*e.g.,* when using potentially infectious sample material). • Sample volume: 50 to 200 µl • Elution volume: 50 to 100 µl [1)]

⚠ [1)] When using whole blood samples, set the elution volume to 100 µl for all purification protocols. Due to the high content of high-molecular weight DNA in whole blood, lower elution volumes may lead to inefficient nucleic acid isolation.

⚠ The Elution Buffer used in the MagNA Pure LC Total Nucleic Acid Isolation Kit contains stabilizing components that interfere with standard OD_{260} measurements.

All "Total NA" purification protocols enable the eluate to be diluted with up to 900 µl Elution Buffer.

Sample Material

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

- 50 to 200 µl mammalian serum or plasma
- 50 to 100 µl mammalian whole blood

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

⚠ Do not use frozen blood, because this could lead to degradation of RNA.

⚠ 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 your WBCs or PBMCs with a hemocytometer before using them in a sample. Note that automatic counting systems (depending on the supplier) sometimes produce cell counts that do not agree with manual hemocytometer counts.

⚠ The "Total NA" purification protocols were developed with human serum, plasma and whole blood. It is important to know 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.

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 Tubs, or similar. All other reagents remaining in the Reagent Tubs after comple-

tion 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 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. ⚠ 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.
Proteinase K	Reconstitute each vial of Proteinase K (vial 5) by first adding 3.0 ml Elution Buffer (bottle 7). Close the vial and mix well, to completely dissolve the lyophilizate. After complete solubilization, add an additional 2.0 ml of the buffer to reach the final volume of 5.0 ml and mix again. 🔍 One vial 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 total nucleic acid samples (*e.g.,* by real-time PCR/RT-PCR assays using 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 enzymebased 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® Carousel-Based 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

General Remarks Pre-isolation steps are required for the "Total NA External_lysis" purification protocol, which includes a manual sample lysis step.

Always freshly prepare lysates and process them immediately.

External Lysis Protocol

❶	Transfer 50 to 200 µl of serum/plasma sample or 50 to 100 µl whole blood sample into a suitable vial, *e.g.,* a reaction tube or the Sample Cartridge.
❷	Add 300 µl Lysis/Binding Buffer (Bottle 4).
❸	Mix the samples thoroughly by gently pipetting up and down.
❹	If necessary, transfer the sample lysate (350 to 500 µl) into the Sample Cartridge.
❺	Place the Sample Cartridge on the Reagent/Sample Stage and start the "Total NA External_lysis" purification protocol, as described in section 2.4.

2.4 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, the software will reduce the volumes of all solutions accordingly (see the 'Start Information' screen of the MagNA Pure LC 1.0 Instrument, or 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 R8. Use Elution Buffer or nuclease-free 10 mM Tris-HCl, pH 8.0 as dilution buffer.

Protocol

Isolate total viral nucleic acids (DNA and RNA) according to the protocol below.

Start Instrument and Software	
MagNA Pure LC 1.0 Instrument	• Turn on the Instrument and the computer, then start the MagNA Pure LC Software. • Navigate to the 'Start Information' screen.
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.
All MagNA Pure LC Instruments	• Select the appropriate protocol:

If you are starting with....	**Then use.....**
unlysed serum, plasma, or whole blood samples	the "Total NA Serum_Plasma_Blood" or the "Total NA Variable_elution_volume" protocol
externally lysed serum, plasma, or whole blood samples	the "Total NA 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	
All MagNA Pure LC Instruments	Before starting the isolation procedure, fill all Reagent Tubs outside the Instrument with the required amount of reagents (equilibrated to +15 to +25°C).
MagNA Pure LC 1.0 Instrument	Fill each Reagent Tub with the volume listed on the 'Start Information' screen, then close it with a Tub Lid.
MagNA Pure LC 2.0 Instrument	Fill each Reagent Tub with the volume listed on the 'Stage Setup' sub-tab, then close it with a Tub Lid.
All MagNA Pure LC Instruments	⚠ 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.

All MagNA Pure LC Instruments	⚠ 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	
MagNA Pure LC 1.0 Instrument	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.
MagNA Pure LC 2.0 Instrument	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.
All MagNA Pure LC Instruments	A colored "Positioning Frame"* that can be placed on the Reagent Reservoir Rack to aid correct loading of the reagents, is available with the MagNA Pure LC Disposables Starter Set.
Load the Samples	
All MagNA Pure LC Instruments	• Transfer the Sample Cartridge, containing the samples or lysates to the MagNA Pure LC Instrument. • Close the Disposable Lockbar.
Start the Batch Run	
MagNA Pure LC 1.0 Instrument	• 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 total viral nucleic acids isolation procedure. The Instrument will automatically dispense all reagents and process the samples.
MagNA Pure LC 2.0 Instrument	• 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 total viral nucleic acids 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/RT-PCR set-up. Do not store the eluted nucleic acid in the MagNA Pure LC Storage Cartridge on Cooling Unit 1.

For storage, close the Storage Cartridge using a MagNA Pure LC Cartridge Seal*, and store the total viral nucleic acids at approx. -70°C (stable for at least several weeks). For long-term storage, it is recommended to store the nucleic acids in aliquots in screw-capped tubes at approx. -70°C. Ensure that the DNA eluates are not be repeatedly frozen and thawed before 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, data may not be reproducible in subsequent assays.

Post Elution Steps

The MagNA Pure LC Instruments can set up RT-PCR, reverse transcription and PCR reactions, by pipetting samples and master mixes (for RT-PCR, RT, or 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 2.0 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

Scalability

To show scalability, Hepatitis A Virus (10^4 – 10^8 copies/ml) or Parvo Virus B19 (10^2 – 10^6 copies/ml) was serially diluted 10-fold to the indicated virus concentrations, in human serum or EDTA-plasma. Two hundred microliters of each sample were purified, using the MagNA Pure LC Total Nucleic Acid Isolation Kit in 2-fold replicates with the MagNA Pure LC Instrument. RT-PCR analysis was then performed using the LightCycler® Carousel-Based System.

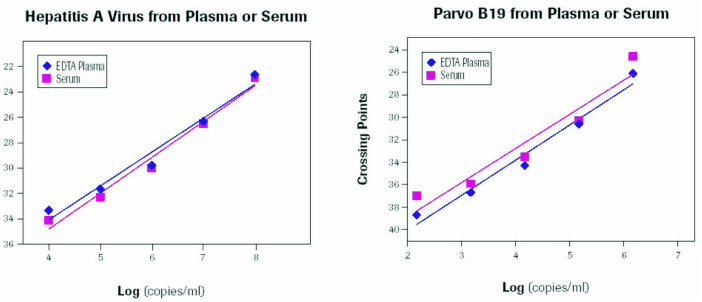


Fig. 1: RT-PCR analysis of Hepatitis A Virus-positive or Parvo Virus B19-positive, human EDTA-plasma or serum samples after purification with the MagNA Pure LC Total Nucleic Acid Isolation Kit, using the LightCycler® Carousel-Based System.

Reproducibility

Different sample materials containing 3.5×10^7 copies/ml of Hepatitis A Virus were processed using the MagNA Pure LC Total Nucleic Acid Isolation Kit in replicates of 30, with the MagNA Pure LC Instrument. To demonstrate reproducibility, eluates were analyzed by RT-PCR analysis, using the LightCycler® Carousel-Based System, targeting HAV (see table 1). The results revealed excellent reproducibility.

Tab.1: Reproducibility shown by HAV-specific RT-PCR analysis using the LightCycler® Carousel-Based System.

Sample	CP [mean]	CV [%]
Plasma (citrate) (50 µl)	26.1	1.8
Serum (50 µl)	26.5	2.0
Whole blood (EDTA) (50 µl)	27.2	2.6
Plasma (citrate) (200 µl)	25.1	1.4
Serum (200 µl)	25.0	0.6
Whole blood (EDTA) (200 µl)	26.8	2.0

Prevention of Cross-Contamination

PCR produces large numbers of copies of a target sequence from minute quantities of DNA - even single copies - for exquisite sensitivity. However, this means that extreme care needs to be taken to avoid generating false-positive results. The laboratory workflow should be in accordance with GLP (Good Laboratory Practice), to prevent cross-contaminations.

Amplicon contamination can occur during sample set-up, the nucleic acid purification, PCR setup and post-PCR processing. Sample materials with high virus titers in particular require a higher precaution during handling. Aerosol formation can play an important role during all processing steps involved. Aerosol formation can lead to sample contamination detectable by low signals in negative control samples.

To avoid negatively impacting results, the following guidelines should be followed:

- Work in a uni-directional manner and carefully plan facility design.
- Define and establish the lower limit of detection for each of PCR application.
- Whenever possible, use UNG (Uracil-DNA Glycosylase, heat-labile*, or LightCycler® Uracil-DNA Glycosylase*), to prevent carryover of PCR amplicons from previous PCRs.
- Always confirm low positive results using an independent assay.

Reproducibility

- **Intra-assay variance:** Total nucleic acids were purified from 30 samples positive for viral RNA and analyzed by RT-PCR on the LightCycler® Carousel-Based System. The CV of the resulting Cps was approx. 3%.
- **Inter-assay variance:** In four independent runs, RNA was purified from 6 samples positive for viral RNA and analyzed by RT-PCR using the LightCycler® Carousel-Based System. The CV of the resulting Cps was approx. 3%.

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 section "Sample Material".
	MGPs were magnetized prior to use.	• Avoid contact between MGPs and magnets • Store kit appropriately
Nucleic acid is degraded.	Storage of samples was not appropriate.	• Use fresh samples, whenever possible. Do not freeze whole blood before processing it. • Avoid the use of samples that have been stored extensively at +15 to +25°C.
	Nuclease contamination of Reaction Tips, Reagent Tubs, Sample Cartridges or reagents.	Avoid contaminating disposables and reagents with nucleases.
Poor nucleic acid purity	Storage of samples was not optimal.	• Use fresh samples, whenever possible. Do not freeze whole blood before processing it. • Avoid the use of samples that have been stored extensively at +15 to +25°C.
	Reagents were placed incorrectly on the Reagent/ Sample Stage.	Ensure that all reagents are in the correct positions on the Reagent/Sample Stage.
	Too much sample material	Reduce amount of sample material to the values indicated in section "Sample Material", or dilute the sample.
Unclear UV spectrum	The Elution Buffer used in the MagNA Pure LC Total Nucleic Acid Isolation Kit contains stabilizing components that interfere with standard $OD_{260\ nm}$ measurements.	Use an alternative measurement method, *e.g.,* quantitation by fluorescent dyes.
Poor PCR performance	Poor purity of nucleic acid	Too much sample material used for isolation, adjust input material to the values indicated in section "Sample Material".
	PCR/RT-PCR reagents and protocols were not optimal.	Check PCR/RT-PCR reagents and protocols with a positive control.
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 the subsequent PCR or RT-PCR using LightCycler® Instruments.

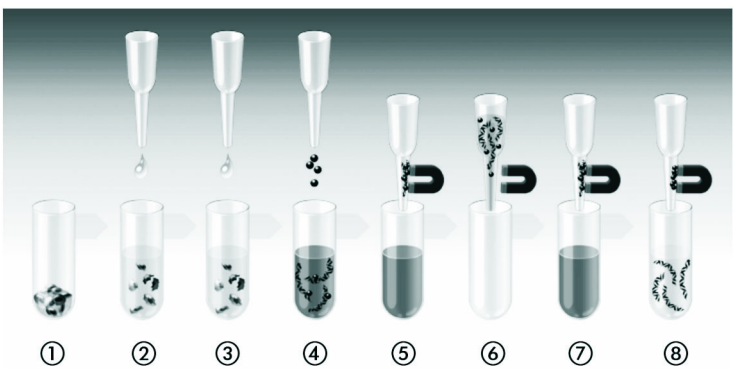
5. Additional Information on this Product

5.1 How this Product Works

The MagNA Pure LC Total Nucleic Acid Isolation Kit is used with the MagNA Pure LC Instruments, to purify high-quality, undegraded total viral nucleic acids (DNA and RNA) from 1 to 32 samples of mammalian serum, plasma, or whole blood. Isolated nucleic acids can be eluted into any volume between 50 and 100 µl. It meets the quality standards required for highly sensitive and quantitative PCR or RT-PCR analysis on the LightCycler® Instruments.

Test Principle

The isolation procedure uses on magnetic-bead technology. Samples are lysed by incubation using a special buffer containing a chaotropic salt and Proteinase K. Magnetic Glass Particles (MGPs) are added and the total viral nucleic acids contained in the sample are bound to their surfaces. Unbound substances are removed by several washing steps, and purified total viral nucleic acids are eluted using a low-salt buffer.
The principle steps of a MagNA Pure LC Total Nucleic Acid 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 and the proteins in the samples are digested.
④ Nucleic acids bind to the silica surface of the added MGPs, due to the chaotropic salt conditions, isopropanol and high ionic strength of the Lysis/Binding Buffer.

⑤	MGPs with bound nucleic acids are magnetically separated from the residual lysed sample.
⑥	MGPs with bound nucleic acids 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 total nucleic acid are magnetically separated from the Wash Buffer containing residual sample debris.
⑧	Purified total viral nucleic acids are 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 Total Nucleic Acid Insolation Kit isolation procedure are as follows:

Sample Lysis	"Total NA External_lysis" protocol only: **Performed manually outside the MagNA Pure LC Instrument**
	Sample is lysed using the Lysis/Binding Buffer, enabling nucleic acid release and nuclease inactivation.
Nucleic Acid Isolation	**Performed using automation by MagNA Pure LC Instruments**
①	Dispense all required reagents into the Processing Cartidge.
②	Dispense Elution Buffer into the Elution Cartridge (Heating Unit).
③	Add Lysis/Binding Buffer to the sample, then mix. (Note: In the "Total NA External_lysis" protocol, the lysate is mixed only.)
④	Transfer lysate into the Proteinase K solution, then mix and incubate.
⑤	Transfer lysate into the MGP suspension, then mix and incubate.
⑥	Transfer MGPs into Wash Buffer I, mix, separate particles.
⑦	Transfer MGPs into Wash Buffer II, mix, separate particles.
⑧	Transfer MGPs into Wash Buffer III, mix, separate particles.
⑨	Transfer MGPs into the Elution Buffer, mix, incubate, elute nucleic acids. Discard MGPs.
⑩	Transfer eluate to the Storage Cartridge (Cooling Unit I).

5.2 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 Serum_Plasma_Blood" 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.

5.3 References

1 Huisman, W. *et al.* (2004). Antibodies specific for hypervariable regions 3 to 5 of the feline immunodeficiency virus envelope glycoprotein are not solely responsible for vaccine-induced acceleration of challenge infection in cats. *J. Gen. Virol.* **85**, 1833-1841.

2 Zhang, Z. *et al.* (2004). Extent of reduction of foot-and-mouth disease virus RNA load in oesophageal-pharyngeal fluid after peak levels may be a critical determinant of virus persistence in infected cattle. *J. Gen. Virol.* **85**, 415-421.

3 Mohammadi, T. *et al.* (2003). Optimization of real-time PCR assay for rapid and sensitive detection of eubacterial 16S ribosomal DNA in platelet concentrates. *J. Clin. Microbiol.* **41**, 4796-4798.

4 Espy, MJ. *et al.* (2002). Detection of smallpox virus DNA by LightCycler PCR. *J. Clin. Microbiol.* **40**, 1985-1988.

5 Loeffler, J. *et al.* (2002). Automated extraction of genomic DNA from medically important yeast species and filamentous fungi by using the MagNA Pure LC System. *J. Clin. Microbiol.* **40**, 2240-2243.

6. Supplementary Information

6.1 Conventions

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

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 Instruction Manual, 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 Instruction Manual, the following abbreviations are used:

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

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 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): http://www.magnapure.com
- Real-time PCR Systems (LightCycler® Carousel-Based System, LightCycler® 480 System and Universal ProbeLibrary): http://www.lightcycler-online.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 2.0 LightCycler® 480 Plate Adapter	1 adapter	05 323 983 001
Positioning Frame	only available with the MagNA Pure LC Disposables Starter Set	03 005 488 001
MagNA Pure LC Cartridge Seals	200 seals	03 118 827 001
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
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

	Product	Pack Size	Cat. No.
Kits for DNA Isolation	MagNA Pure LC DNA Isolation Kit I	1 kit (192 isolations)	03 003 990 001
	MagNA Pure LC DNA Isolation Kit II (Tissue)	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
	MagNA Pure LC DNA Isolation Kit – Large Volume	1 kit (96 to 288 isolations)	03 310 515 001
Kits for RNA 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
Kits for Total Nucleic Acid Isolation	MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume	1 kit (192 isolations)	03 264 793 001
	MagNA Pure LC Total Nucleic Acid Isolation Kit - High Performance	1 kit (96 to 288 isolations)	05 323 738 001
Associated Reagents	Uracil DNA Glycosylase, heat-labile	100 U 500 U	11 775 367 001 11 775 375 001
	LightCycler® Uracil DNA Glycosylase	100 U (50 µl)	03 539 806 001

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