For general laboratory use.

MagNA Pure LC Total Nucleic Acid Isolation Kit

Version: 18

Content Version: October 2020

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 1 kit
up to 192 isolations

Store the kit at +15 to +25 °C

1. General Information

1.1. Contents

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

The bottles of the Washbuffer 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.

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

Additional Lysis/Binding Buffer (bottle 4) is available for preparing large numbers of sample lysates for external lysis by obtaining the MagNA Pure LC Total Nucleic Acid Isolation Kit - Lysis/Binding Buffer Refill.

1.2. Storage and Stability

Storage Conditions (Product)

Unopened kit components of the MagNA Pure LC Total Nucleic Acid Isolation Kit are stable at +15 to +25°C until the expiration date printed on the label.

Storage Conditions (Working Solution)

Vial / Bottle	Cap	Label	Preparation	Storage
6	black	Magnetic Glass Particles (MGPs) Suspension	The MGP suspension 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.***
5	pink	Proteinase K	Reconstitute each vial of Proteinase K 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.

1.3. Additional Equipment and Reagent required

- MagNA Pure LC 2.0 Instrument

Standard laboratory equipment:
- Pipettes and nuclease free, aerosol-preventive tips to pre-dispense samples into the sample cartridge
- Centrifuge and suitable nuclease free reaction tubes
- Vortex mixer

1.4. 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 2.0 Instrument, 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.

Product Description

Kit for isolation of total viral nucleic acids from mammalian serum, plasma and whole blood, using MagNA Pure LC Instruments. The kit is designed for 192 isolations (6 runs with 32 samples) from up to 200 µl mammalian serum or plasma, or up to 100 µl mammalian whole blood.

1.5. Preparation Time

Assay Time

- MagNA Pure LC Instrument setup: 15 minutes total
- 90 minutes when processing 1 to 32 samples, using either the 'Total NA Serum_Plasma_Blood' or 'Total NA Variable_elution_volume' purification protocols.

ⓘ *No hands-on time is required after setup 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

Sample Materials

For optimal results in downstream procedures, such as real-time PCR with LightCycler® 480 and Carousel-Based Instruments, do not process larger sample volumes or cell numbers than described below. Too much purified total nucleic acid will cause the MGPs to clump and reduce total nucleic acid yield from the purification run.

Different mammalian species will have concentrations of blood cells that are significantly different from human blood. For some species, lower blood volumes will be required to remain under the 1x10 cell number limit.

For best results, use fresh or fresh frozen samples. Avoid the use of samples that have been stored at RT (+15 to +25°C). Whole blood may be stored at RT 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.

The maximum amount of sample material for use with the MagNA Pure LC Total Nucleic Acid Isolation Kit is:

- 50 to 200 µl mammalian serum or plasma
- 50 to 200 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 × 1x10^6 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 × 1x10^6 blood cells), always count your WBCs or PBMCs with a hemocytometer before using them in a sample. Please 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.***

Control Reactions

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® 480 or Carousel-Based 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 www.lightcycler.com.

General Considerations

Handling requirements

- 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.
- Do not pool reagents from different lots or from different bottles of the same lot.

Prevention of Carryover Contamination

The ability of PCR to produce large numbers of copies from just a single copy of a given target sequence offers the ultimate in sensitivity. For this reason, extreme care must be taken to avoid the detection of false-positive results due to cross-contamination of samples during either sample preparation, the purification run, or PCR or RT-PCR setup.

For best and most consistent results and to prevent cross contaminations, the laboratory workflow should follow guidelines that are in accordance with GLP (Good Laboratory Practice). Cross contamination can occur during sample transfer and lysis, nucleic acid purification, and PCR set up. Cross-contamination can be due either to direct exchange of material between two samples or due to previously amplified DNA found in the laboratory environment such as benchtop, gloves, pipetting devices, disposables and glassware.

Sample materials with potentially high virus titers must be handled with the utmost care to both prevent cross contamination and direct contact with laboratory personnel. Always treat all samples as potentially infectious. Aerosol formation can play an important role during all processing steps involved the workflow. Aerosol formation can lead to sample contamination which can be detected by low signals in negative control samples usually found in the range of the lower detection limit of a specific assay.

In order to best evaluate the significance of positive and negative findings, use the following recommendations as a guideline:

- Work in an unidirectional manner and carefully design the location and ordering of the workflow. If possible, carry out sample preparation, purification runs and PCR in three separate rooms.
- Always include a positive and negative control in each phase of the workflow.
- Always use PCR-dedicated pipetting devices with reaction tips that have aerosol-barriers.
- Define and establish the lower limit of detection for each kind of PCR application, and always confirm low-end positive results using an independent experiment.

Uracil-DNA Glycosylase (UNG) prevents contamination by PCR carryover

Whenever possible use the enzyme uracil-DNA glycosylase (UNG), either LightCycler® Uracil-DNA Glycosylase for FastStart Polymerases, and Uracil-DNA Glycosylase, heat-labile for non-FastStart Polymerases, to prevent carryover of PCR amplicons from previous PCRs. For details please refer to the intruction for use for the two UNG products named above.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis/Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease-free pipet tips only, to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.
- Complete each phase of the PCR/RT-PCR workflow before proceeding to the next phase. For example, you should finish PCR/RT-PCR sample preparation before starting PCR/RT-PCR set-up. Sample preparation, PCR/RT-PCR set-up and the PCR/RT-PCR run itself should also be performed in separate locations.

Waste handling

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

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.

Working Solution

Preparation of Working Solutions

Before starting the procedure, prepare the **the Magnetic Glass Particles and Proteinase K working solutions** *described below.*

All other solutions are ready to use. The Lysis/Binding Buffer (bottle 4) is blue to enable the MagNA Pure LC Instrument to detect reaction tips that have blocked tips (clot detection). All other buffers are transparent. Do not use a buffer when it contains a precipitate. If a precipitate is present, place the bottle at +37°C and mix it until the precipitate is completely dissolved.

For most reproducible results, 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 room temperature.

Bring all buffers to room temperature (+15 to +25°C) before use. If you use the reagents at temperatures outside this recommended range, a less favorable and less reproducible purification outcome could result.

To conserve the reagents in this kit, use only the volumes of reagent required by the number of samples being processed by the MagNA Pure LC Instrument.

Never store the Proteinase K and the MGPs Suspensions in reagent tubs. All other reagents remaining in the reagent tubs after completion of the run can be used for the next run if that next run is performed on the same day. Longer storage periods in the reagent tubs are not recommended due to evaporation and the resulting changes in reagent volumes and concentrations that affect run-to-run reproducibility.

Magnetic Glass Particles (MGPs)

Each of the six vials of MGPs Suspension (Reagent 6) must be mixed thoroughly before use to produce a homogeneous suspension for pipetting into the reagent tub. The MGPs sediment quickly during storage. For best and most consistent results, always add the MGPs to the instrument **just before** starting the run to minimize sedimentation. Always use the volume of MGPs recommended by the MagNA Pure LC Software.

Never leave the MGPs Suspension in its vial without tightening the cap, or in the reagent tub without both the reagent tub lid and seal. Please note that ethanol evaporation will lead to suboptimal and less reproducible run-to-run purification outcomes.

Preparation of Proteinase K solution

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

Please note that after dissolving, the Proteinase K solution may appear turbid. This is caused by stabilizing components present in the Proteinase K lyophilizate, and has no impact on either the pipetting or functionality of the Proteinase K solution.

2.2. Protocols

Purification Protocols

- The MagNA Pure LC Instruments are designed to process 32 samples at the same time. If you are processing fewer samples, reduce the volumes of all solutions accordingly by using the start information screen of the MagNA Pure LC Software 3.0 as a guide.
- The software automatically calculates the necessary amounts of reagents and guides you through the setup.
- Note that the instrument cannot be started until the interlock for securing sample cartridge, reagent tubs, and reaction tips is closed.
- When the option to program the dilution of the eluate is selected, be certain that an additional reagent tub M30 is placed in position R8. Use either Elution Buffer or nuclease-free 10 mM Tris-HCl, pH 8.0 as the dilution buffer.

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

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

Protocol Name	Sample Materials	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.

Pre-Isolation Steps

The isolation of total nucleic acids from serum and plasma samples proceeds in fully automated handsfree manner when using the 'Total NA Serum_Plasma_Blood' and 'Total NA Variable_elution_volume' protocols. For these protocols, no pre-isolation steps are required. For the 'Total NA External_lysis' protocol, carry out the manual lysis steps described below.

External lysis protocol

Pre-isolation steps are required for the 'Total NA External_lysis' purification protocol, which includes a manual sample lysis step. The protocol was developed with human blood, serum or plasma.

Always freshly prepare lysates and process them immediately.

1. Transfer 50 to 200 µl of serum/plasma sample or 50 to 100 µl whole blood sample into a suitable vial, such as reaction tubes or the sample cartridge.
2. Add 300 µl Lysis/Binding Buffer (Bottle 4).
3. Mix the samples thoroughly by pipetting.
4. If necessary, transfer the sample lysate (350 to 500 µl) into the Sample Cartridge
5. Place the Sample Cartridge on the Reagent/Sample Stage and start the "Total NA External_lysis" purification protocol, as described in the Total Nucleic Acid Isolation Protocol.

Total Nucleic Acid Isolation Protocol

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

	MagNA Pure LC 2.0 Instrument
Start Instrument and Software	▪ 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 with unlysed serum, plasma, or whole blood samples, then use the "Total NA Serum_Plasma_Blood" or the "Total NA Variable_elution_volume" protocol. If you are starting with externally lysed serum, plasma, or whole blood samples, then use the "Total NA External_lysis" protocol.
	▪ Follow the instructions of the Software and specify the name and number of samples. ▪ Type in the appropriate Sample Volume, Elution Volume and Dilution Volume (if necessary). The software will calculate how much of each reagent is required to carry out these steps.
Fill the Reagent Tubs	▪ For best results and convenience, always fill the reagent tubs outside the instrument with the required amount of reagents warmed to room temperature. ▪ Fill each reagent tub with the volume listed on the start information screen, then cover it with the appropriate tub lid
	▪ 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.*** ▪ For a delay in starting the purification run, always seal off the reagent tub lids with the reagent tub lid seals. ▪ Please note that the sealed tubs are not completely airtight and thus 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.
	ℹ *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	▪ 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 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 robot in the MagNA Pure LC Instrument is ideal for setting up PCR reactions. Simply program the instrument using the post-elution MagNA Pure LC software, to pipet the purified DNA samples and master reagent mixes for PCR into either LightCycler® Capillaries, standard PCR tubes or 96-well plates. It is possible to program the post-elution steps either before you performing the isolation procedure for seamless post-elution pipetting or after it is complete.

For automated post-elution pipetting for LightCycler® Carousel-Based Instruments, place the appropriate number of empty LightCycler® Capillaries in either the MagNA Pure LC Cooling Block for LC Centrifuge Adaptors or the MagNA Pure LC Cooling Block for the LC Sample Carousel. For LightCycler® 480 Instruments, place the MagNA Pure LC Cooling Block for 96-well PCR Plates that is used to hold LightCycler® 480 Multiwell Plates. For details, please see the MagNA Pure LC Instrument Operator's Manual for the best MagNA Pure LC cooling blocks for your downstream applications. Contact your local Roche Representative for how to best set up your 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.

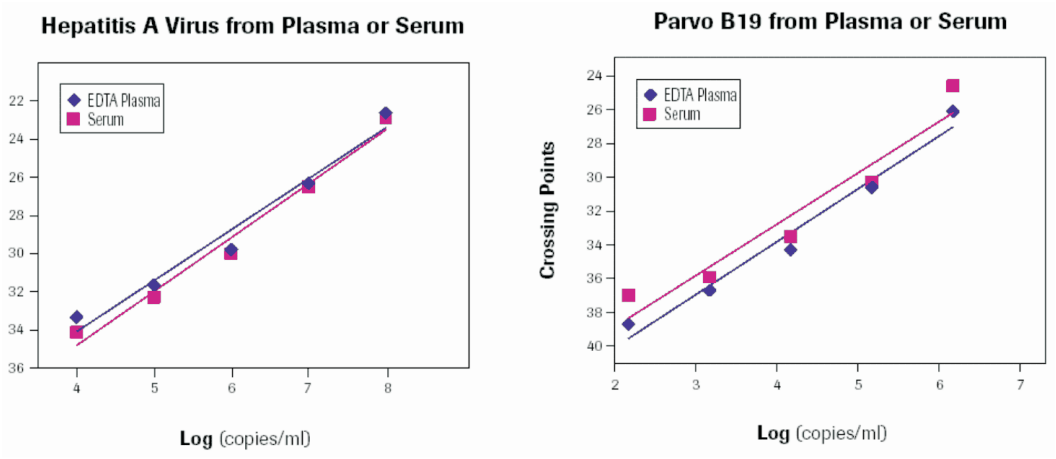


Fig. 1: RT-PCR analysis of Hepatitis A Virus-positive or Parvo Virus B19-positive, human EDTA-plasma or serum samples after purification with the MagNA Pure LC Total Nucleic Acid Isolation Kit, using the LightCycler® Carousel-Based System.

Reproducibility

- Intra-assay variance: A variance of approx. 3% was found in the LightCycler® System's Cps obtained from 30 samples (n = 30) in a single purification run from purified total nucleic acid positive for viral RNA and analyzed by LightCycler® RT-PCR.
- Inter-assay variance: A variance of approx. 3% was found in the LightCycler® System's Cps obtained from 6 samples in four independent purification runs (n = 4) from purified total nucleic acid positive for viral RNA and analyzed by LightCycler® System's RT-PCR.

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.

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

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

4. Troubleshooting

Observation	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 materia	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 OD260 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.***

⚠ ***The red color does not affect real-time PCR assays on LightCycler® 480 and LightCycler® Carousel-Based Instruments.***

5. Additional Information on this Product

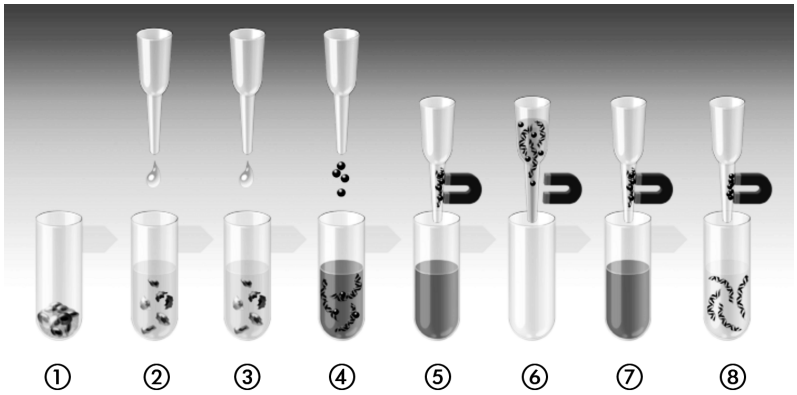
5.1. Test Principle

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.

Isolation Procedure

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 is "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 is performed by 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 MagNA Pure LC Total Nucleic Acid Isolation Kit is function tested by isolating viral nucleic acid from Hepatitis A Virus (HAV)-positive and Parvo Virus B19-positive human reference material using the 'Total NA Serum_Plasma_Blood' protocol. Purified viral HAV and a Parvo Virus B19-specific nucleic acid was detected using real-time PCR with the LightCycler®System. Kit components are also tested to show the absence of nucleases according to the current quality control procedures.

6. Supplementary Information

6.1. Conventions

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

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

6.2. Changes to previous version

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 full overview of related products and manuals, please visit and bookmark our homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
Accessories general (hardware)		
LC Carousel Centrifuge 2.0		12 189 682 001
	1 centrifuge plus rotor and rotor bucket (115 V)	03 709 507 001
	1 centrifuge plus rotor and rotor bucket (230 V), *Not available in US*	03 709 582 001
MagNA Pure LC Cooling Block, LC Centrifuge Adapters	1 cooling block	12 190 664 001
MagNA Pure LC 2.0 LightCycler® 480 Plate Adapter	1 adapter	05 323 983 001
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
Consumables		
LightCycler® 480 Multiwell Plate 96, white	5 x 10 plates	04 729 692 001
MagNA Pure LC Cartridge Seals	200 seals	03 118 827 001
MagNA Pure LC Disposable Starter Set	1 set	03 005 488 001
LightCycler® 480 Multiwell Plate 96, clear	5 x 10 plates	05 102 413 001
LightCycler® Capillaries (20 µl)	5 x 96 capillaries, containing 5 boxes, each with 96 capillaries and stoppers, 5 boxes, each with 96 capillaries and stoppers	04 929 292 001
Instruments		
LightCycler® 480 Instrument II	1 instrument	05 015 278 001
	1 instrument	05 015 243 001
Reagents, kits		
Uracil-DNA Glycosylase, heat-labile	100 U, 1 U/µl	11 775 367 001
	500 U, 1 U/µl	11 775 375 001
LightCycler® Uracil-DNA Glycosylase	50 µl, 100 U, (2 U/µl)	03 539 806 001

6.4. Trademarks

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

6.5. License Disclaimer

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

6.6. Regulatory Disclaimer

For general laboratory use.

6.7. Safety Data Sheet

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

6.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.
To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

Visit **lifescience.roche.com**, to download or request copies of the following Materials:
- Instructions for Use
- Safety Data Sheets
- Certificates of Analysis
- Information Material

To call, write, fax, or email us, visit **lifescience.roche.com** and select your home country to display country-specific contact information.

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