For general laboratory use.



# MagNA Pure LC Total Nucleic Acid Isolation Kit – High Performance

🔃 Version: 13

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

Cat. No. 05 323 738 001 1 kit up to 288 isolations

Store the kit at +15 to +25°C

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# 1. General Information

### 1.1.Contents

Vial / Bottle	Сар	Label	Function	Content
1	black	Wash Buffer I	for removal of PCR inhibitors	3 bottles, 100 ml each
2	blue	Wash Buffer II	for removal of salts, proteins etc.	3 bottles, 64 ml each
3	red	Wash Buffer III	for removal of salts etc.	2 bottles, 100 ml each
4	green	Lysis/Binding Buffer	for lysis and binding of total nucleic acid	1 bottle, 100 ml
5	pink	Proteinase K	for digestion of proteins	3 glass vials, lyophilizate
6	clear	Proteinase K Buffer II	for reconstitution of Proteinase K	1 bottle, 100 ml
7	black	Magnetic Glass Particles (MGPs) Suspension	for binding of total nucleic acid	6 vials, 11 ml MGP suspension each
8	yellow	Elution Buffer	for elution of pure total nucleic acid	1 bottle, 100 ml
			for dilution of eluates (optional)	_

*i* 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.

### 1.2. Storage and Stability

### **Storage Conditions (Product)**

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

### **1.3. Additional Equipment and Reagents Required**

- MagNA Pure LC 1.0 Instrument
- MagNA Pure LC 2.0 Instrument

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, to resuspend the MGPs

### 1.4. Application

The MagNA Pure LC Total Nucleic Acid Isolation Kit - High Performance is used with the MagNA Pure LC 2.0 Instrument to isolate highly purified total viral nucleic acids (DNA and RNA) from 1 to 32 samples of mammalian serum, plasma, or whole blood. Purified total nucleic acid can be used both in PCR or RT-PCR on the LightCycler<sup>®</sup> Instruments or standard thermal block cyclers. Purified total nucleic acids are free of PCR inhibitors according to our quality control procedures.

The MagNA Pure LC Total Nucleic Acid Isolation Kit - High Performance is used for automated isolation of total viral nucleic acids from up to 1,000  $\mu$ l mammalian serum, plasma or whole blood. The kit is designed for 288 isolations from 100  $\mu$ l and 200  $\mu$ l or 192 isolations from 500  $\mu$ l and 96 isolations from 1,000  $\mu$ l mammalian plasma, serum or blood. The isolated nucleic acids can be eluted in 50 or 100  $\mu$ l.

*i* The kit is designed to process up to 288 samples in batches of 32 (depending on the protocol). If you process fewer than 32 samples at a time, some reagent will be wasted and the remaining reagent may not be enough to process the number of samples listed above.

# 1.5. Preparation Time

Setup of the MagNA Pure LC Instruments requires approximately 15 min. Total time for the automated purification of total nucleic acid from 32 samples, depends on the choice of protocol:

Protocol	Sample volume	Run time	Comment
Total NA HP 200	100 or 200 µl	90 min	For standard applications
Total NA HS 200	100 or 200 µl	180 min	For maximum recovery and sensitivity
Total NA HP 200 External_Lysis	100 or 200 µl	90 min	For external lysis / standard applications
Total NA HS 200 External_Lysis	100 or 200 µl	180 min	For external lysis / maximum recovery and sensitivity
Total NA HS 500	500 µl	140 min	For maximum recovery and sensitivity
Total NA HS 500 External_Lysis	500 µl	140 min	For external lysis / maximum recovery and sensitivity
Total NA HS 1000	1,000 µl	180 min	For maximum recovery and sensitivity

1 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**

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

- 100 or 200 µl mammalian serum or plasma<sup>1)</sup> or blood<sup>1)</sup> for the 100 or 200 µl volume protocols
- 500 μl mammalian serum or plasma<sup>1)</sup> or blood<sup>1)</sup> for the 500 μl volume protocols
- 1,000 µl mammalian serum or plasma<sup>1)</sup> or blood<sup>1)</sup> for the 1,000 µl volume protocols
- <sup>(1)</sup> Plasma or blood containing EDTA or citrate as anticoagulant may be used.
- Vever 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.
- The Total NA HP and Total NA HS purification protocols were developed with human serum, plasma and whole blood. Remember that different mammalian species may have different concentrations of blood cells. For some species, you may need to use a smaller sample of blood to keep the cell numbers within the above guidelines. Blood collected from different blood donors may contain different concentrations of blood cells. If you expect extremely high blood cell counts in a sample, use less (e.g., 100 µl instead of 200 µl) or dilute the sample, (e.g., with PBS).

#### A Treat all samples as potentially infectious.

### **Control Reactions**

Always run appropriate controls with the samples, especially when performing quantification analyses with the eluted total nucleic acid samples on the LightCycler<sup>®</sup> 480 or LightCycler<sup>®</sup> Carousel-Based Instruments.

Monitor both, the process of sample preparation and subsequent downstream applications by using the following control sample materials:

- · Positive control using a sample material that is known to be positive for your target.
- Negative control using a sample material that is known to be negative for your target.
- Internal controls (IC) using specially-designed control templates. The Internal Control (IC) is produced by adding a defined amount of control template such as plasmid DNA to samples to be purified using the MagNA Pure LC Instrument.
- *i* The IC is added prior to the purification step and then co-purified and amplified with your target of interest in the same PCR reaction. The IC concept is especially useful for enzyme-based amplification processes such as PCR, because efficiency of the PCR process may be reduced by inhibitors present in the purified sample material. In addition, the IC is used to compensate for possible losses of your target during purification.
- For quantification assays using LightCycler<sup>®</sup> 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<sup>®</sup> Carousel-Based System, read the LightCycler<sup>®</sup> 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 hazardous irritants. Do not allow the Wash Buffer I or Lysis/Binding Buffer to come in contact with skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If necessary, immediately contact your laboratory supervisor, and seek medical assistance. When spilling these reagents, dilute the spill with large amounts of water before attempting to clean up the spill.
- Do not allow the above two buffers to mix with sodium hypochlorite (bleach) solution or strong acids. This mixture can produce a highly toxic gas.
- Never pool reagents from different MagNA Pure LC reagent lots or from different bottles of the same lot.

### **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 setup 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).

### **Working Solution**

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

### **Magnetic Glass Particles (MGPs)**

Store MGPs at +15 to +25°C.

- A 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 and less reproducible run-to-run purification outcome.

The MGP suspension (vial 7) must be mixed thoroughly. Vortex immediately before use to produce a homogeneous suspension. The beads tend to sediment during storage.

A 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.

### Proteinase K

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. Reconstitute each vial of Proteinase K (vial 5) by first adding 4.0 ml Proteinase K Incubation Buffer II (vial 6). Close the vial and mix well, to completely dissolve the lyophilizate. After complete solubilization, add an additional 2.7 ml of the buffer to reach the final volume of 6.7 ml and mix again.

All other solutions are ready-to-use. The Lysis/Binding Buffer contains a blue ingredient required for clot detection during automated nucleic acid isolation by the MagNA Pure LC Instruments. 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 best 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 +15 to +25°C.

Equilibrate all buffers to +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 Instruments.

▲ Never store the Proteinase K and the MGPs suspensions in reagent tubs. All other reagents remaining in the MagNA Pure LC 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-torun reproducibility.

### 2.2. Protocols

### **Pre-Isolation Steps**

Pre-isolation steps are required for the '*Total NA HP 200 External\_Lysis*', the '*Total NA HS 200 External\_Lysis*' and the 'Total NA HS 500 External\_Lysis' purification protocols, which include a manual sample lysis step.

Always freshly prepare lysates and process them immediately. Store the lysate at -60 to -80°C when nucleic acid isolation is postponed.

#### **External lysis protocol**

Transfer 100 μl, 200 μl or rather 500 μl of serum/plasma/blood sample into a suitable vial, *e.g.*, a reaction tube or the sample cartridge.

Add 300 µl Lysis/Binding Buffer (bottle 4) for the 'Total NA HP 200 External\_Lysis' protocol or the 'Total NA HS 200 External\_Lysis' protocol or 450 µl Lysis/Binding Buffer for the 'Total NA HS 500 External\_Lysis' protocol. Mix the samples thoroughly by gently pipetting up and down.

3 If necessary, transfer the sample lysate into the sample cartridge.

Place the sample cartridge on the reagent/sample stage and start either the 'Total NA HP 200 External\_ Lysis' protocol or the 'Total NA HS 200 External\_Lysis' protocol or rather the 'NA HS 500 External\_Lysis' protocol.

### **Purification Protocols**

To perform total viral nucleic acid isolations (DNA and RNA) with the MagNA Pure LC Total Nucleic Acid Isolation Kit - High Performance, new purification protocols must be installed. 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. If not previously installed, order the protocols free of charge. For assistance, contact your local Roche representative. Use the following table to determine which protocol is best for your sample material or application.

Protocol Name	Sample Material	Procedure
Total NA HP 200	100 μl or 200 μl serum or plasma or blood	<ul> <li>Fully automated.</li> <li>Elution volume: 50 or 100 μl<sup>1)</sup></li> <li>For standard applications.</li> </ul>
Total NA HS 200		<ul> <li>Fully automated.</li> <li>Elution volume: 50 or 100 μl<sup>1)</sup></li> <li>For maximum recovery and sensitivity.</li> </ul>
Total NA HP 200 External_Lysis and Total NA HS 200 External_Lysis		<ul> <li>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.</li> <li>Enables the physical separation of the lysis step from the purification step and to load inactivated sample material into the MagNA Pure LC Instruments (<i>e.g.</i>, when using potentially infectious sample material)</li> <li>Elution volume: 50 or 100 µl<sup>1</sup>)</li> </ul>
Total NA HS 500	500 µl serum or plasma or blood	<ul> <li>Fully automated.</li> <li>Elution volume: 50 or 100 μl<sup>1)</sup></li> <li>For maximum recovery and sensitivity.</li> </ul>
Total NA HS 500 External_Lysis		<ul> <li>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.</li> <li>Enables the physical separation of the lysis step from the purification step and to load inactivated sample material into the MagNA Pure LC Instruments (<i>e.g.</i>, when using potentially infectious sample material)</li> <li>Elution volume: 50 or 100 µl<sup>1</sup>)</li> </ul>
Total NA HS 1000	1,000 µl serum or plasma or blood	<ul> <li>Fully automated.</li> <li>Elution volume: 50 or 100 μl<sup>1)</sup></li> <li>For maximum recovery and sensitivity.</li> </ul>

<sup>(1)</sup> Choosing 50 µl elution volume rather than 100 µl does not result in exactly double the sensitivity, because the elution efficiency is lower with a smaller volume.

*i* All Total NA HP and Total NA HS purification protocols enable the eluate to be diluted with up to 900 μl Elution Buffer.

#### **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 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 setup.
- You cannot 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.

MagNA Pure Instrument Version	1.0	2.0	Step		
Start Instrument and	x		Turn on the instrument and the computer, then start the MagNA Pure LC Software.		
Software			Navigate to the 'Start Information' screen.		
		x	Turn on the instrument, the MagNA Pure LC 2.0 Software starts automatically. Log in and then navigate to the ' <i>Ordering</i> ' sub-tab.		
	x	Х	Select the appropriate protocol:		
	x	x	If you are starting Then use with		
			unlysed 100 - 200 µl either the ' <i>Total NA HP 200</i> ' or the ' <i>Total NA HS 200</i> ' serum, plasma, or whole protocol blood samples		
			externally lysed 100 - either the ' <i>Total NA HP 200 External_Lysis</i> ' protocol 200 µl serum, plasma, or or the ' <i>Total NA HS 200 External_Lysis</i> ' protocol whole blood samples		
			unlysed 500 µl serum, the ' <i>Total NA HS 500</i> ' protocol plasma, or whole blood samples		
		externally lysed 500 µl the ' <i>Total NA HS 500 External_Lysis</i> ' protocol serum, plasma, or whole blood samples			
			unlysed 1,000 µl serum, the ' <i>Total NA HS 1000</i> ' protocol plasma, or whole blood samples		
	x	х	Follow the instructions in 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	x	х	Before starting the isolation procedure, fill all reagent tubs outside the instrument with the required amount of reagents (equilibrated to $+15$ to $+25$ °C).		
	x		Fill each reagent tub with the volume listed on the 'Start Information' screen, then close it with a tub lid.		
		х	Fill each reagent tub with the volume listed on the ' <i>Stage Setup</i> ' sub-tab, then close it with atub lid.		
	x	x	<ul> <li>Close reagent tubs with the tub lids, to prevent evaporation of the reagents. Even when closed, reagent tubs are not suitable for long-term storag of reagents.</li> <li>Load the exact amount of MGPs (as listed on the 'Start Information' screen or 'Stage Setup' sub-tab) on to the tnstrument. Load the MGPs just before starting the run to prevent them from sedimenting.</li> </ul>		

# Total Nucleic Acid Isolation - HighPerformance Protocol using the MagNA Pure LC Instruments

MagNA Pure Instrument Version	1.0	2.0	Step
Set up reagent tubs and disposables on	x		Use the information of the ' <i>Start Information</i> ' screen to place all disposable plastics and reagents within reagent tubs, necessary for the batch run on the reagent/sample stage.
the reagent/ sample stage		х	Use the information of the ' <i>Stage Setup</i> ' sub-tab to place all disposable plastics and reagents within reagent tubs, necessaryfor the batch run on the reagent/ sample stage.
	х	x	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	x x	Transfer the sample cartridge, containing the samples or lysates to the MagNA Pure LC Instrument.	
			Close the disposable lockbar.
Start the batch run	atch x		On the ' <i>Start Information</i> ' screen, confirm the correct placement of all disposable plastics and reagents, by mouse-clicking the respective text boxes.
			Click the ' <i>OK</i> ' button, to start the automated total viral nucleic acids isolation procedure. The instrument will automatically dispense all reagents and process the samples.
		x	On the ' <i>Stage Setup</i> ' 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 ' <i>Start</i> ' 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 setup. Do not store the eluted nucleic acids in the MagNA Pure LC Storage Cartridge on Cooling Unit 1.

For storage, close the storage cartridge with a MagNA Pure LC Cartridge Seal\*, and store the total viral nucleic acids at -60 to  $-80^{\circ}$ C (for at least several weeks). For long-term storage, store aliquots of the nucleic acid in screw-capped tubes at -60 to  $-80^{\circ}$ C, so that the solutions are not 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. When nucleic acids are not premixed and distributed evenly/ homogenously in solution, results 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<sup>®</sup> Capillaries, standard PCR tubes or plates. See the MagNA Pure LC Instrument Operator's Manuals for recommended plates. For post elution procedures, you can place LightCycler<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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 Instrument Operator's Manuals, for details on how to set up a post elution run.

### **2.3. Other Parameters**

### **Prevention of Carryover Contamination**

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

# 3. Results

The MagNA Pure LC Total Nucleic Acid Isolation Kit - High Performance was used with the MagNA Pure LC Instrument to purify DNA Virus (CMV, EBV) and RNA Virus (HAV, InfA) positive human samples, purifying nucleic acids with high specificity and sensitivity for PCR and RT-PCR.

Each preparation was used as template in PCR (DNA) or RT-PCR (RNA). All these templates produced specific PCR/RT-PCR products with reproducable yield.

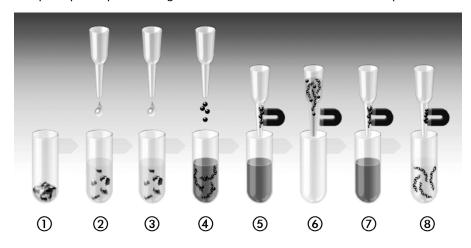
# 4. Troubleshooting

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 prior to use. Store kit appropriately.
Nucleic acid is	Storage of samples was not appropriate.	Use fresh samples, whenever possible.
degraded		Avoid the use of samples that have been stored extensively at $+15$ to $+25^{\circ}$ C.
	Nuclease contamination of Reaction tips, reagent tubs, sample cartridges or reagents.	Avoid contaminating disposables and reagents with nucleases.
Poor or no nucleic	Storage of samples was not optimal.	Use fresh samples, whenever possible.
acid yield		Avoid the use of samples that have been stored extensively at $+15$ to $+25^{\circ}$ C.
	Reagents were placed incorrectly on the reagent/sample stage.	Ensure that all reagents are in the correct positions on the Reagent/ Sample Stage.
Poor nucleic acid purity	Too much sample material or too many blood cells in the sample.	Reduce amount of sample material to the values indicated in section "Sample Material", or dilute the sample.
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	<ul> <li>Centrifuge at low g-values (approx. 1,000 rpm) to remove fines.</li> <li><i>i</i> The red color does not affect PCR or RT-PCR on LightCycler<sup>®</sup> 480 and LightCycler<sup>®</sup> Carousel-Based Instruments.</li> </ul>

# 5. Additional Information on this Product

### 5.1. Test Principle

The isolation procedure is based on magnetic-bead technology. The samples are lysed by incubation with a special buffer containing a chaotropic salt and Proteinase K. Magnetic glass particles (MGPs) are added and total viral nucleic acids contained in the sample are bound to their surfaces. Unbound substances are removed by several washing steps, then the purified total viral nucleic acids are eluted with 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 Isolation Kit - High Performance isolation procedure on the MagNA Pure LC Instruments are as follows:

(9) Dispense all required reagents into the processing cartridge.

10 Dispense elution buffer into the elution cartridge (heating unit).

(1) Add lysis/binding buffer to the sample, then mix. Total viral nucleic acids are released and nucleases are inactivated.

#### A This step is omitted in the External\_lysis Protocols.

- (12) Transfer lysate into Proteinase K solution, then mix and incubate.
- (13) Transfer lysate into MGP suspension, then mix and incubate.
- (14) Transfer MGPs into wash buffer I, mix, separate particles.
- (15) Transfer MGPs into wash buffer II, then mix, separate particles.
- (16) Transfer MGPs into wash buffer III, then mix, separate particles.
- (17) Transfer MGPs into the elution buffer (heating unit), mix, incubate, elute nucleic acids.
- (18) Separate and discard MGPs.
- (19) Transfer eluate to the storage cartridge (cooling unit I).
- *i* For external-lysis protocols only: Perform sample lysis manually outside the MagNA Pure LC Instrument. Sample is lysed using the lysis/binding buffer, enabling nucleic acid release and nuclease inactivation.

### **5.2. Quality Control**

- The kit is function tested by isolation of total viral nucleic acids (DNA and RNA) from human reference material using the Total NA HP 200 purification protocol. The purified total viral nucleic acids are then detected by quantitative, real-time PCR and RT-PCR using virus-specific assays established for the LightCycler<sup>®</sup> System.
- The kit components are tested for 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						
<i>i</i> Information Note: Add	<i>i</i> Information Note: Additional information about the current topic or procedure.					
Important Note: Infe	<b>Important Note: Information critical to the success of the current procedure or use of the product.</b>					
1 2 3 etc.	Stages in a process that usually occur in the order listed.					
1 2 3 etc.	Steps in a procedure that must be performed in the order listed.					
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.					

### 6.2. Changes to previous version

Layout changes Editorial changes

# 6.3. Ordering Information

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

Product	Pack Size	Cat. No.
Accessories general ( hardware )		
LC Carousel Centrifuge 2.0	1 centrifuge plus rotor and rotor bucket (115 V)	03 709 507 001
	1 centrifuge plus rotor and rotor bucket (230 V)	03 709 582 001
MagNA Pure LC Cooling Block, LC Centrifuge Adapters	1 cooling block	12 190 664 001
MagNA Pure LC Cooling Block, LC Sample Carousel	1 cooling block	12 189 704 001
MagNA Pure LC 2.0 LightCycler <sup>®</sup> 480 Plate Adapter	1 adapter	05 323 983 001
MagNA Pure LC Cooling Block, 96-well PCR Plate	1 cooling block	12 189 674 001
Consumables		
MagNA Pure LC Cartridge Seals	200 seals	03 118 827 001
LightCycler <sup>®</sup> 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
LightCycler <sup>®</sup> 480 Multiwell Plate 96, white	5 x 10 plates	04 729 692 001
LightCycler <sup>®</sup> 480 Multiwell Plate 96, clear	5 x 10 plates	05 102 413 001
MagNA Pure LC Disposable Starter Set	1 set	03 005 488 001
Instruments		
LightCycler <sup>®</sup> 2.0 Instrument	1 instrument	03 531 414 001
LightCycler <sup>®</sup> 480 Instrument II	1 instrument	05 015 278 001
	1 instrument	05 015 243 001
MagNA Pure LC 2.0 Instrument	1 instrument	05 197 686 001

### 6.4. Trademarks

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### 6.5. License Disclaimer

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# 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

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- Safety Data Sheets

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