For general laboratory use.

MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi)

Version: 18

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Kit for isolation of bacterial and fungal DNA from difficult-to-process sample materials using the MagNA Pure LC Instruments

Cat. No. 03 264 785 001 1 kit
up to 192 isolations

Store the kit at +15 to +25°C.

1. General Information ... 3
1.1. Contents ... 3
Storage Conditions (Product) ... 4
1.2. Additional Equipment and Reagent required ... 4
1.3. Application ... 4
1.4. Preparation Time ... 4
Assay Time ... 4
2. How to Use this Product ... 5
2.1. Before you Begin ... 5
Sample Materials ... 5
Control Reactions ... 5
General Considerations ... 6
Precautions ... 6
Number of Samples ... 6
Safety Information ... 6
Laboratory Procedures ... 6
Waste Handling ... 6
Working Solution ... 7
2.2. Protocols ... 8
Purification Protocol ... 8
Pre-Isolation Steps ... 8
Special Sample Materials ... 9
DNA Isolation Protocol ... 10
Storage of DNA Eluates ... 11
Post Elution Steps ... 11
3. Results ... 12
BAL Samples ... 12
Urine Samples ... 12
Stool Samples ... 13
Bacterial Cultures ... 14
Fungi ... 14
Other Sample Materials ... 14
4. Troubleshooting ... 15
5. Additional Information on this Product ... 16
5.1. Test Principle ... 16
Automated DNA Isolation Using the MagNA Pure LC Instruments ... 17
5.2. Quality Control ... 17
6. Supplementary Information ... 18
6.1. Conventions ... 18
6.2. Changes to previous version ... 18
6.3. Ordering Information ... 19
6.4. Trademarks ... 20
6.5. License Disclaimer ... 20
6.6. Regulatory Disclaimer ... 20
6.7. Safety Data Sheet (SDS) ... 20
6.8. Contact and Support ... 20

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	black	MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi), Wash Buffer I	To remove PCR inhibitors.	2 bottles, 100 ml each
2	blue	MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi), Wash Buffer II	To remove salts, proteins etc.	2 bottles, 64 ml each
3	red	MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi), Wash Buffer III	To remove residual salts.	1 bottle, 100 ml
4	green	MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi), Lysis/Binding Buffer	For cell lysis and DNA binding.	1 bottle, 100 ml
5	black	MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi), Magnetic Glass Particles	For DNA binding.	6 vials, MGP suspension
6	yellow	MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi), Elution Buffer	▪ For elution of pure DNA. ▪ Reconstitution of Proteinase K. ▪ For dilution of eluates (optional).	1 bottle, 100 ml
7	clear	MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi), Bacteria Lysis Buffer	For efficient bacterial cell lysis.	2 bottles, 30 ml each
8	pink	MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi), Proteinase K	For protein digestion.	6 glass vials, Lyophilized

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

Wash Buffer I (Bottle 1) and the Magnetic Glass Particles (MGPs, Bottle 5) have black caps, however the color-coding of the MagNA Pure LC Software and Positioning Frames refer to a caramel cap for the MGPs.

Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	black	Wash Buffer I	Store at +15 to +25°C.
2	blue	Wash Buffer II	
3	red	Wash Buffer III	
4	green	Lysis/Binding Buffer	
5	black	Magnetic Glass Particles	
6	yellow	Elution Buffer	
7	clear	Bacteria Lysis Buffer	
8	pink	Proteinase K	

1.2. Additional Equipment and Reagent required

Standard Laboratory Equipment

- Pipettes and nuclease-free, aerosol-resistant tips to predispense samples into the MagNA Pure LC Sample Cartridge
- Centrifuge and suitable nuclease-free reaction tubes
- Vortex mixer to resuspend the MGPs
- Heating device (+65 to +95°C, for reaction tubes: 1.5 to 2 ml)

1.3. Application

For general laboratory use. The MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi), a General Purpose Reagent (GPR), is designed for use with the MagNA Pure LC 2.0 Instrument to isolate highly purified bacterial and fungal DNA from a variety of sample materials. Purified DNA can be used for real-time PCR using LightCycler® Instruments* or standard thermal block cyclers.

1.4. Preparation Time

Assay Time

MagNA Pure LC Instrument Setup	approximately 15 minutes
Automated purification of DNA from 32 samples	approximately 95 minutes

No hands-on time is required after set up of the MagNA Pure LC Instruments. Additional 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, especially in real-time PCR assays using LightCycler® Instruments, do not process samples larger than this kit is designed to handle.

- Optimal amount of sample material: 100 µl liquid sample

> ⚠ ***Do not use more sample material than indicated. This may affect the performance of the isolation process and may lead to clumping and loss of MGPs, or cross-contamination of samples.***

Follow these general guidelines when preparing samples:

- Treat all samples as potentially infectious.
- If starting from larger volumes of liquid samples (e.g., 0.5 to 1 ml, or more), or samples with low or unknown bacterial load (e.g., urine, CSF, BAL, aspirates), centrifuge samples for 10 minutes at 8,000 × g to concentrate the bacterial cells in the pellet. Discard most of the supernatant, leaving a final volume of 50 to 100 µl, and process the concentrate.
- If you assume a liquid sample is very cell-rich (e.g., it appears turbid), it may be wise to use less sample, thus limiting the amount of DNA obtained. This will make the purified DNA more suitable for common downstream assays. For example, too much eukaryotic genomic DNA can overload a PCR assay.
- The best way to prepare a semi-liquid sample (BAL, sputum, CSF, stool, etc.) for DNA isolation will depend greatly on the type of sample material, sample viscosity, or content of particles. Due to the great variety of materials, no single, generally applicable procedure can be given. Be prepared to vary or optimize the described procedures as needed. For example, additional lysis steps may increase DNA recovery from some bacterial species.
- To process “special sample materials” (stool, biopsies, swabs), see section **Protocols**.

Control Reactions

Always run appropriate controls with the samples, especially if you want to perform quantification analyses of the eluted DNA samples using real-time PCR assays with the LightCycler® Instruments.
The following control procedures are recommended:

- Positive Control, using a sample material positive for your target.
- Negative Control, using a sample material negative for your target.
- Extraction Control, using water.
- Internal Control (IC), by adding a defined amount of a control template (e.g., plasmid DNA) to all samples to be purified.

Use Water, PCR Grade for the Extraction Control.*

The IC is added prior to the purification step and then co-purified and amplified with your target of interest in the same PCR reaction. The IC concept is especially useful for enzyme-based amplification processes such as PCR, because efficiency of the PCR process may be reduced by inhibitors present in the purified sample material. In addition, the IC is used to compensate for possible losses of your target during purification.

For quantification assays on the LightCycler® Instruments, use a synthetic double-stranded DNA molecule with primer-binding sites identical to those of your target sequence, but having a unique probe-binding region that differentiates the IC from the target-specific amplicon. Discriminate the signals derived from your target and the IC by performing a dual-color HybProbe Assay. For detailed information regarding the IC concept, in combination with the LightCycler® 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

Precautions

- Wash Buffer I (Bottle 1) and Lysis/Binding Buffer (Bottle 4) contain guanidinium salts which are hazardous irritants.

  ⚠ ***Do not allow them 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. For spilled reagents, dilute the spill with large amounts of water before wiping it up.***

- Do not allow Wash Buffer I (Bottle 1) or the Lysis/Binding Buffer (Bottle 4) to come in contact with sodium hypochlorite (bleach) solution.

  ⚠ ***This mixture can produce a highly toxic gas.***

- Do not pool reagents from different MagNA Pure LC reagent lots or from different bottles of the same lot.
- Do not use the kit after the expiration date.

Number of Samples

The MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi) is designed to process up to 192 samples in six batches of 32 from up to 100 µl of liquid samples. When processing fewer than 32 samples at a time, more reagent will be used up and lost in dead volume during each run and the remaining reagents will not be enough to process 192 samples.

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 pipettes and nuclease-free pipette tips only to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.
- Finish 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 setup. Perform sample preparation, PCR/RT-PCR setup, and the PCR/RT-PCR run itself in separate locations.

Waste Handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online at dialog.roche.com, or upon request from the local Roche office.

Working Solution

Before starting the procedures, prepare the working solutions as described below:

Reagent	Preparation	Storage and Stability
Magnetic Glass Particles (Vials 5)	▪ Mix the MGP suspension (Vials 5) 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 at +15 to +25°C. ▪ Do not store the MGP suspension in a Reagent Tub or similar container. ▪ Do not leave the MGP suspension uncovered in the vial or in the Reagent Tub as evaporation may lead to suboptimal purification.
Proteinase K (Vials 8)	▪ Reconstitute each vial of Proteinase K (Vial 8) by adding 1.2 ml Elution Buffer (Bottle 6). ▪ Close the vial and mix well to completely dissolve the lyophilizate. *After dissolving, the Proteinase K solution may appear turbid. This is caused by stabilizing components added to the Proteinase K. This appearance has no impact on the functionality of the enzyme.* ▪ One vial of Proteinase K is sufficient for 32 samples.	Store 1 month at +2 to +8°C, or up to 12 months at -15 to -25°C.

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 occasionally until the precipitate is completely dissolved. Stop heating the buffer as soon as the precipitate is completely dissolved. 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 the number of samples.

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

2.2. Protocols

Purification Protocol

To perform DNA isolations with the MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi), one purification protocol is available. The protocol name listed below should appear on the 'Ordering' sub-tab of the MagNA Pure LC 2.0 Instrument.

Protocol Name	Sample Material	Procedure
DNA III Bacteria	50 to 100 µl liquid sample	▪ Depending on the sample type, pre-isolation treatment may be necessary to liquify the sample. The liquid sample is then transferred to the MagNA Pure LC Reagent/Sample Stage. ▪ DNA purification is performed automatically by the Instrument. ▪ Sample volume: 50 to 100 µl ▪ Elution volume: 100 µl

The "DNA III Bacteria" purification protocol enables the eluate to be diluted with up to 900 µl Elution Buffer.

Pre-Isolation Steps

① Liquefaction is an optional step for very viscous sample material such as BAL and sputum.
- Prepare a fresh DTT* stock solution (e.g., 5x conc. = 0.75%).
- Adjust the final concentration of DTT in the sample to 0.15% by adding appropriate amounts of DTT stock solution.
- Incubate the sample, for example, by shaking for 30 minutes at +37°C until it can be easily pipetted.

② Centrifugation is an optional step for large-volume liquid samples that have low or unknown bacterial loads, such as urine, CSF, BAL, or aspirates.
- Centrifuge the sample for up to 10 minutes at 8,000 × *g* to concentrate the bacterial cells in the pellet.
- Discard most of the supernatant, leaving only 50 to 100 µl; then resuspend the cells in the remaining liquid and process the concentrate.

This centrifugation step enables a dilute sample that originally contains several milliliters of liquid to be processed in a single isolation, ensuring maximum DNA yield.

③ Addition of Bacteria Lysis Buffer.
- Add 130 µl of Bacteria Lysis Buffer (Bottle 7) to 100 µl of sample.

⚠ ***If the sample volume is less than 100 µl, add enough Bacteria Lysis Buffer to make a final volume of 230 µl.***
- Mix well.

⚠ ***If you intend to perform the MagNA Lyser Homogenization Step (see Step 7), increase the volume of both the sample and the Bacteria Lysis Buffer to 250 µl each. The final volume of Bacteria Lysis Buffer plus sample is 500 µl.***

④ Enzymatic Digestion is an optional step to enhance lysis of certain bacteria, such as Gram-positive species.
- Depending on the type of bacteria to be lysed, prepare one of the following enzyme cocktails:

Enzyme Cocktail	Contents	Function
I	N-acetylmuramidase (0.625 mg/ml = 2,500 U/ml) and beta-1,3-glucanase (Zymolase) (0.25 mg/ml = 500 U/ml); dissolved in 50% glycerol/50 mM Tris-acetate, pH 6.7.	Lyses a broad spectrum of Gram-positive bacteria.
II	Lysozyme (100 mg/ml = 50 kU/ml) and lysostaphin (5 mg/ml = 5 kU/ml); dissolved in 5% glycerol/PBS, pH 7.5.	Especially useful for lysis of *Staphylococcus spec.*

- Add 5 µl per 100 µl total sample volume of the enzyme cocktail to the Bacteria Lysis Buffer/sample mixture.

You can premix Enzyme Cocktail and Bacteria Lysis Buffer and add them both to the sample in a single step.
- Incubate at +37°C for 10 to 30 minutes.

⑤ Proteinase K Digestion
- Add 20 µl of Proteinase K to the mixture and mix thoroughly.

⚠ ***If you do not use an enzyme cocktail, premix Proteinase K and Bacteria Lysis Buffer and add them to the sample in a single step.***

⚠ ***If you intend to perform the MagNA Lyser Homogenization Step (see Step 7), increase the volume of both the sample and Bacteria Lysis Buffer/Proteinase K to 250 µl each. The final volume of Bacteria Lysis Buffer plus Proteinase K plus sample is 500 µl.***

- Incubate for 10 minutes at +65°C (e.g., BALs) or overnight at +15 to +25°C (e.g., biopsies, solid tissue), until the sample completely disintegrates.

⑥ Boiling

ⓘ *Perform this step to inactivate potentially pathogenic organisms in the sample. It may also enhance lysis of the cell wall of some bacterial species. To prevent leakage, perform this step in screw-capped reaction tubes.*
- Incubate samples at +95°C for 10 minutes.
- Centrifuge briefly to collect the entire sample volume at the bottom of the tube.
- Allow samples to cool down or chill on ice; then transfer the cooled sample to the Sample Cartridge.

⑦ MagNA Lyser Treatment is an optional mechanical homogenization to enhance lysis of some bacterial species, especially those that are Gram-positive. It is also very useful for samples that contain particles, such as stool.
- Transfer up to 500 µl sample into a MagNA Lyser Green Beads* tube.
- Place the tube into the MagNA Lyser Instrument*.
- Homogenize for 30 seconds at 6,000 rpm.
- Cool samples for 1 minute in the MagNA Lyser Rotor Cooling Block*.
- Centrifuge for 5 minutes at 17,000 × *g* at +15 to +25°C.
- Transfer 100 to 250 µl of the lysate supernatant into the Sample Tube.

ⓘ *For some sample types, you may improve DNA yield by performing this homogenization step first, before adding Bacteria Lysis Buffer.*

Special Sample Materials

Stool

❶ Take an appropriate, peanut-sized stool sample and resuspend in PBS to a final volume of approximately 500 µl (1:5 dilution).

⚠ ***Do not centrifuge.***

❷ Remove 100 µl of the stool suspension and add 130 µl Bacteria Lysis Buffer (Bottle 7) and 20 µl Proteinase K (Vial 8).

❸ Incubate for 10 minutes at +65°C.
- Incubate an additional 10 minutes at +95°C.

⚠ ***To avoid clogging Reaction Tips with solid particles, let the lysate sediment or centrifuge at 500 × g. Alternatively, homogenize the lysate for 30 seconds at 6,000 rpm in the MagNA Lyser Instrument, then centrifuge at 500 × g.***

❹ Allow samples to cool down or chill on ice.

❺ Transfer up to 100 µl cooled and clarified supernatant to the Sample Cartridge.

Biopsies

❶ To a 1 to 10 mg piece of tissue, add 130 µl Bacteria Lysis Buffer (Bottle 7) and 20 µl Proteinase K (Vial 8).

❷ Incubate at +65°C until the sample is completely disintegrated.
- Incubate for 10 minutes at +95°C.

❸ Allow samples to cool down or chill on ice.

❹ Transfer up to 100 µl cooled lysate to the Sample Cartridge.

Swabs

1. Submerge swab tip in 130 µl Bacteria Lysis Buffer (Bottle 7) and 20 µl Proteinase K (Vial 8).
2. Incubate for 10 minutes at +65°C.
3. Squeeze the liquid from the swab and collect the liquid.
   - Heat this lysate for 10 minutes at +95°C.
4. Allow samples to cool down or chill on ice.
5. Transfer up to 100 µl cooled liquid to the Sample Cartridge.

Blood Culture

1. To 100 µl of blood culture medium, add 130 µl Bacteria Lysis Buffer (Bottle 7) and 20 µl Proteinase K (Vial 8).
2. Incubate for 10 minutes at +65°C.
   - Incubate an additional 10 minutes at +95°C.
3. Allow samples to cool down or chill on ice.
4. Transfer up to 100 µl cooled lysate to the Sample Cartridge.

Fungi

1. To increase the lysis efficiency for fungi and spore-forming bacteria, perform 3 to 5 “freeze/boil cycles” using liquid nitrogen and a heating block after Proteinase K digestion.
2. Transfer up to 100 µl cooled liquid to the Sample Cartridge.

DNA Isolation Protocol

This 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 ‘**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. The Disposable Lockbar, for securing the Sample Cartridge, Reagent Tubs, and Reaction Tips must be closed to start the Instrument. If you programmed dilution of the eluate, you will need an additional Reagent Tub M30 in position R7. Use Elution Buffer (Botlle 6) or nuclease-free 10 mM Tris-HCl, pH 8.0 as dilution buffer.

Protocol for the Isolation of Bacterial and Fungal DNA

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 protocol "DNA Bacteria III". ▪ Follow the instructions of the Software and specify the name and number of samples. Type in the Sample Volume, Elution Volume, and Dilution Volume (if necessary). The software will calculate how much of each reagent is required.
Fill the Reagent Tubs
Before starting the isolation procedure, fill all Reagent Tubs outside the Instrument with the required amount of reagents equilibrated to +15 to +25°C.
Fill each Reagent Tub with the volume listed on the 'Stage Setup' sub-tab.
▪ Close Reagent Tubs with the Tub Lids to prevent evaporation of the reagents. *Reagent Tubs are not suitable for long-term storage of reagents.* ▪ Load the exact amount of MGPs (as listed on the 'Stage Setup' sub-tab) on to the Instrument just before the run starts. This will prevent them from sedimenting.
Set Up Reagent Tubs and Disposables on the Reagent/Sample Stage
Use the information of the 'Stage Setup' sub-tab to place all disposable plastics and reagents within Reagent Tubs, necessary for the batch run on the Reagent/Sample Stage.
A colored "Positioning Frame"* that can be placed on the Reagent Reservoir Rack to aid correct loading of 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 DNA isolation procedure. The Instrument will automatically dispense all reagents and process the samples.

Storage of DNA Eluates

- Close the Storage Cartridge with a MagNA Pure LC Cartridge Seal* and store the DNA at -15 to -25°C for at least several weeks.
- For long-term storage, store the DNA in aliquots in screw-capped tubes at -15 to -25°C.

⚠ ***Avoid repeated freezing and thawing.***

- To ensure greatest possible stability of the eluted nucleic acids, immediately proceed with PCR setup.
- Do not store the eluted nucleic acid in the MagNA Pure LC Storage Cartridge on Cooling Unit 1.
- After thawing eluates, mix gently by pipetting up and down ten times before performing any downstream steps, such as RT-PCR, or OD measurements. If nucleic acids are not premixed and distributed homogeneously in solution, results may not be reproducible in subsequent assays.

Post Elution Steps

The MagNA Pure LC Instruments set up PCR reactions by pipetting DNA samples and master mixes for PCR into either LightCycler® Capillaries* or 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 into 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 finished.
See the MagNA Pure LC Operator's Manuals for details on how to set up a Post Elution run.

3. Results

Typical results obtained using the MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi) with the standard protocol are shown below. The efficiency of the procedure depends on the nature of the sample. Since some samples are very viscous, cell-rich, or contain inhibitory substances, results vary. Modifications of the standard protocol may be necessary to obtain satisfactory results, see section **Pre-Isolation Steps**.

BAL Samples

BAL sample material shows significant variations in viscosity and cell content. However, even very viscous samples can be processed using the described pre-treatment, see section **Pre-Isolation Steps**. Six different BAL samples of varying viscosity were spiked with *S. aureus* (a typical organism found in such samples). After DNA isolation, all samples were positive using PCR analysis on the LightCycler® Carousel-Based System (Fig. 1). BAL samples with very high viscosity or high load of human cells showed higher crossing point (Cp) values, indicating a suboptimal recovery. In such cases, reducing the amount of input sample or extending the Proteinase K step, can help to ensure correct processing and maximal sensitivity. In contrast, a BAL sample that was found positive for *S. aureus* by microbiological testing showed a significantly lower Cp, indicating that the total number of *S. aureus* in this particular sample was higher than spiked amounts (Fig. 1).

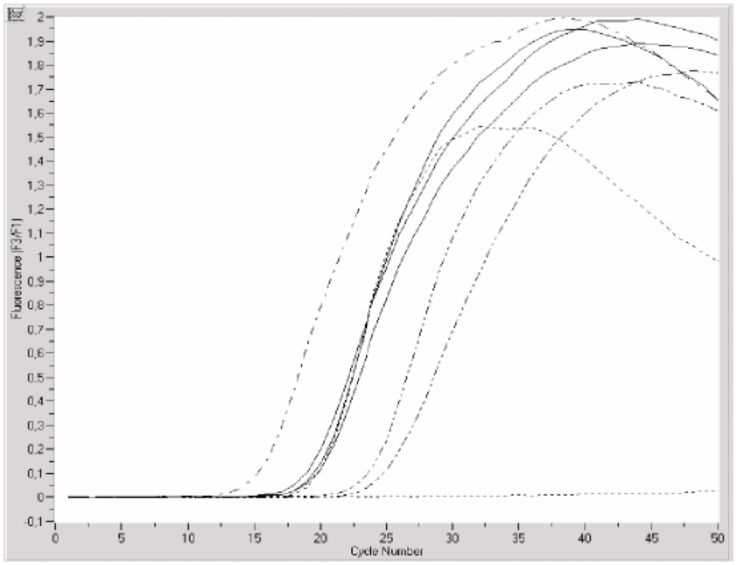


Fig. 1: PCR analysis on the LightCycler® Carousel-Based System of DNA from 6 different BAL samples spiked with S. aureus.
------- BAL positive for S. aureus by microbiological testing with significantly lower Cp
____ Clear or slightly viscous BALs,_._._._ very viscous BALs.....Positive and negative controls

Urine Samples

Nine different urine samples were spiked with cultured *S. aureus* organisms and DNA was isolated as described, see section **Pre-Isolation Steps**. Two microliters of each eluate were analyzed in a specific real-time PCR assay using the LightCycler® Carousel-Based System, together with a dilution series of standard DNA. In all urine samples, a positive PCR signal was obtained. Comparison of the Cps of the samples with those of the standards indicated a high recovery (Fig. 2).

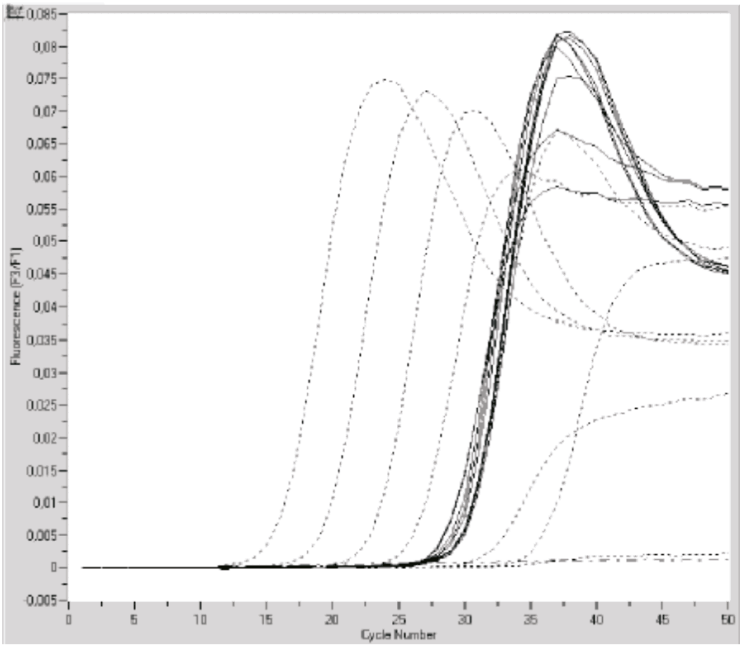


Fig. 2: PCR analysis using the LightCycler® Carousel-Based System of nine different urine samples spiked with S. aureus, compared to a dilution series of standard DNA.
.....Standards_____ Urine samples_._._._._ Negative control

Stool Samples

Three different stool samples were spiked with cultured *H. pylori*, in duplicates. DNA was isolated using the MagNA Pure LC Instrument as described, see section **DNA Isolation Protocol**, and 2 µl of each eluate were analyzed in a *H. pylori*-specific PCR assay with the LightCycler® Carousel-Based System. For all samples, a positive PCR signal was obtained (Fig. 3).

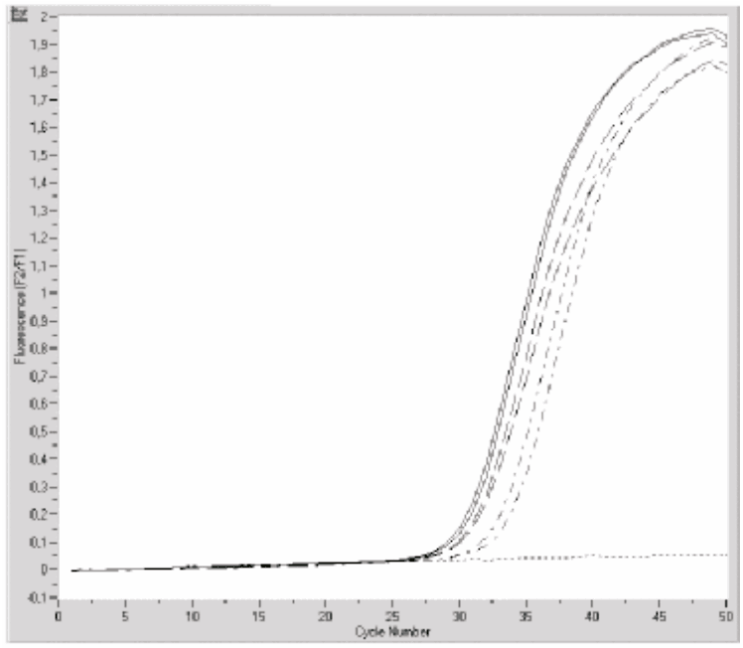


Fig. 3: PCR analysis using the LightCycler® Carousel-Based System of DNA from three different stool samples spiked with cultured H. pylori organisms and isolated with the MagNA Pure LC Instrument.
_____ Stool sample 1 (in duplicates)_._._._._Stool sample 2 (in duplicates)
---------- Stool sample 3 (in duplicates).....Negative control

Bacterial Cultures

Cultures of *Enterococcus faecalis*, *Escherichia coli*, *Corynebacterium striatum*, *Legionella pneumophila*, *Streptococcus agalactiae (GBS)*, *Bordetella pertussis*, *Chlamydia pneumoniae,* and a variety of other Gram-positive and Gram-negative bacterial species were used for DNA isolation. DNA isolated using the MagNA Pure LC Instrument, with the MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi) could be detected with a sensitivity that was similar to or higher to the sensitivity achieved for DNA isolated using filter tube methods (data not shown).

Fungi

Both *Aspergilus fumigatus* and *Candida albicans* were efficiently isolated from spiked BALs. PCR results using DNA isolated with the MagNA Pure LC Instrument were equal to, or better than those from parallel experiments using DNA isolated by filter tube methods (data not shown). The sensitivity was significantly increased by subjecting samples to 3 to 5 “freeze/boil cycles” using liquid nitrogen and a heating block, prior to transfer to the MagNA Pure LC Instrument.

Other Sample Materials

Other sample materials such as swabs, biopsies, sputum, CSF, tracheal secretion, and certain blood culture media were processed using the MagNA Pure LC Instrument, and their eluates transferred and successfully analyzed by PCR analysis using the LightCycler® Carousel-Based System (data not shown).

4. Troubleshooting

Observation	Possible cause	Recommendation
Clumping of beads or presence of beads in Storage Cartridge.	Too much sample material, sample too viscous or inefficiently liquefied.	Reduce amount of sample material to the values recommended in the section **Sample Material**.
		Use recommended liquefying method, see section **Pre-Isolation Steps**, and optionally extend incubation time with DTT and Proteinase K.
	MGPs were magnetized prior to use.	Avoid contact between MGPs and magnets prior to use.
		Store kit properly.
DNA is degraded.	Samples not stored properly.	Use fresh or frozen samples whenever possible.
		Avoid using samples stored extensively at +15 to +25°C.
Poor DNA yield.	Sample does not contain enough material.	Centrifuge samples prior to use, then use pellet, see sections **Sample Material** and **Pre-Isolation Steps**.
	Storage of samples was not optimal.	Use fresh or frozen samples whenever possible.
		Avoid using samples stored extensively at +15 to +25°C.
	Too much sample material.	Use 100 µl or less for cell-rich samples per isolation.
	Inefficient lysis	Use recommended lysis protocol including boiling step and optionally extend the Proteinase K step.
Poor DNA purity.	Too much sample material (cell-rich).	Reduce amount of sample material to the values recommended in section **Sample Material**.
Poor PCR performance.	Too much DNA in PCR.	Check DNA concentration in the DNA eluates and adjust amount of eluate per PCR or dilute the eluate 1:10. *For best results, use 1 to 100 ng per PCR maximum: approximately 1 µg.*
		Use less starting material for DNA isolation. Optimal results are obtained with no more than 100 µl of liquid sample.
	Not enough DNA in PCR.	Check DNA concentration of eluates and adjust amount of eluate per PCR, or use more starting material. *Optimum: 1 to 100 ng/PCR, maximum: approximately 1 µg.*
		Use more starting material.
		Centrifuge to concentrate sample, see sections **Sample Material** and **Pre-Isolation Steps**.
	Poor purity of DNA.	Use less sample material for DNA isolation and optionally extend Proteinase K digestion step.
	PCR reagents and protocols were not optimal.	Check PCR reagents and protocols with a positive DNA control (e.g., human genomic DNA*).
	PCR conditions are not optimal.	Optimize PCR, for example, include an initial denaturation of 30 seconds to 3 minutes in the PCR program.
Eluates show a slight red color.	Minimal abrasion from magnetic particles.	Centrifuge at low *g*-values (approximately 1,000 rpm) to remove fines. ***The red color does not affect PCR on the LightCycler® Instruments.***

5. Additional Information on this Product

5.1. Test Principle

The isolation procedure is based on the magnetic-bead technology (Fig. 4). Samples are lysed by incubation with a special buffer containing a chaotropic salt and Proteinase K. Magnetic Glass Particles (MGPs) are added and the DNA is bound to their surfaces. Unbound substances are removed by several washing steps, and purified DNA is eluted using a low-salt buffer.

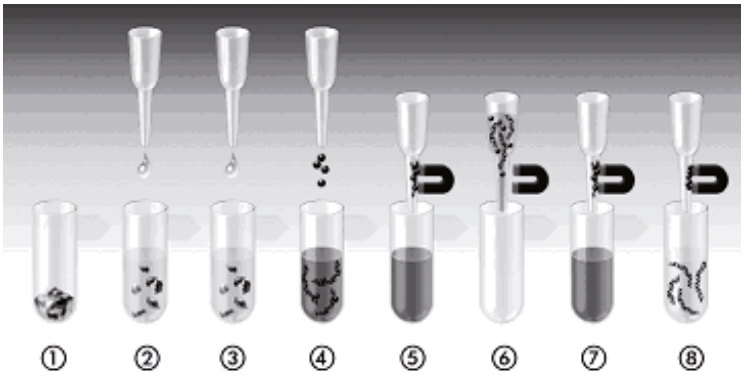


Fig. 4: Principle steps of a MagNA Pure LC DNA isolation.

① Sample material is placed into the wells of the Sample Cartridge.

② Lysis/Binding Buffer is added to the sample, resulting in full cell lysis and release of nucleic acids.
– Nucleases are denatured.

③ Proteinase K is added to the samples and proteins are digested.

④ DNA binds to the silica surface of the added MGPs due to the chaotropic salt conditions, isopropanol, and the high ionic strength of the Lysis/Binding Buffer.

⑤ MGPs with bound DNA are magnetically separated from the residual lysed sample.

⑥ MGPs with bound DNA are washed repeatedly with Wash Buffer to remove unbound substances, (e.g., proteins [nucleases], cell membranes, and PCR inhibitors such as heparin or hemoglobin), and to reduce the chaotropic salt concentration.

⑦ MGPs with bound DNA are magnetically separated from the Wash Buffer containing residual sample debris.

⑧ Purified DNA is eluted from the MGPs in the wells of the Elution Cartridge.
– MGPs are retained in the Reaction Tip and discarded.

Automated DNA Isolation Using the MagNA Pure LC Instruments

The basic steps of the MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi) isolation procedure are shown below:

① Dispense all required reagents into the Processing Cartridge.

② Dispense Elution Buffer into the Elution Cartridge (Heating Unit).

③ Add Lysis/Binding Buffer to the sample and mix; nucleic acid release and nuclease inactivation.

④ Transfer lysate into MGP suspension, then mix and incubate.

⑤ Transfer MGPs into Wash Buffer I, mix, and separate particles.

⑥ Transfer MGPs into Wash Buffer II, mix, and separate particles.

⑦ Transfer MGPs into Wash Buffer III, mix, and separate particles.

⑧ Transfer MGPs into the Elution Buffer (Heating Unit), mix, incubate, and elute DNA.
– Discard MGPs.

5.2. Quality Control

DNA is isolated from urine spiked with defined numbers of *E. coli* using the standard protocol, and analyzed with respect to DNA integrity, recovery, purity, and ability to amplify using the LightCycler®System.

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

Layout changes.
Editorial changes.
Update of quality control.

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)		
MagNA Lyser Rotor Cooling Block	1 rotor cooling block	03 359 085 001
MagNA Pure LC Cooling Block, LC Sample Carousel	1 cooling block	12 189 704 001
MagNA Pure LC 2.0 LightCycler® 480 Plate Adapter	1 adapter	05 323 983 001
MagNA Pure LC Cooling Block, LC Centrifuge Adapters	1 cooling block	12 190 664 001
MagNA Pure LC Cooling Block, 96-well PCR Plate	1 cooling block	12 189 674 001
Consumables		
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
MagNA Pure LC Disposable Starter Set	1 set	03 005 488 001
MagNA Pure LC Cartridge Seals	200 seals	03 118 827 001
Instruments		
LightCycler® 2.0 Instrument	1 instrument	03 531 414 001
MagNA Lyser Instrument	1 instrument, 110 V	03 358 968 001
	1 instrument, 220 V	03 358 976 001
Reagents, kits		
Human Genomic DNA	100 µg, 500 µl	11 691 112 001
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
1,4-Dithiothreitol	custom fill	10 197 785 103
	2 g	10 197 777 001
	10 g	10 708 984 001
	25 g	11 583 786 001
MagNA Lyser Green Beads	100 tubes, prefilled with ceramic beads	03 358 941 001

6.4. Trademarks

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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 (SDS)

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.

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