For general laboratory use.

MagNA Pure LC DNA Isolation Kit II (Tissue)

Version: 18

Content Version: October 2020

Kit for isolation of genomic DNA from mammalian tissues using MagNA Pure LC Instruments

Cat. No. 03 186 229 001 1 kit
up to 192 isolations

Store the kit at +15 to +25°C.

1. General Information

1.1. Contents

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

Wash Buffer I (Bottle 1) and the Magnetic Glass Particles (MGPs, Vial 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.

1.2. 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.
The kit is shipped at +15 to +25°C.

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	Tissue Lysis Buffer	
8	pink	Proteinase K	

1.3. 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)
- Homogenization device:
  - MagNA Lyser Instrument* with MagNA Lyser Green Beads*
  - Rotor-stator homogenizer, such as Ultra Turrax or Omni TH 220
  - Mortar/pestle/needle (0.8 mm)

Deparaffinization

- Hemo-De or xylol
- Ethanol, absolute

Stabilization Reagent

- RNA*later*

1.4. Application

For general laboratory use. The MagNA Pure LC DNA Isolation Kit II (Tissue), a General Purpose Reagent (GPR), is designed for use with the MagNA Pure LC 2.0 Instrument to isolate highly purified genomic DNA from mammalian tissues. Purified DNA can be used for real-time PCR with LightCycler® Instruments or standard thermal block cyclers.

1.5. Preparation Time

Assay Time

MagNA Pure LC 2.0 Instrument Setup	approximately 15 minutes
Automated purification of DNA from 32 samples	approximately 100 minutes for fresh-frozen tissue samples.
	approximately 60 minutes for deparaffinized sections of formalin-fixed, paraffin-embedded, and difficult to homogenize tissue samples (requires an external Proteinase K digestion).

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:

- 1 to 10 mg fresh-frozen or unfrozen, stabilized (e.g., treated with RNA*later*) tissue (depending on the tissue type).
- 1 formalin-fixed, paraffin-embedded tissue section (5 to 10 µm) thick.

⚠ ***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.***
⚠ ***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 DNA samples using real-time PCR assays with 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.
- 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 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), Lysis/Binding Buffer (Bottle 4), and Tissue Lysis Buffer (Bottle 7) 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 II (Tissue) is designed to process up to 192 samples in six batches of 32 from up to 1 to 10 mg fresh-frozen tissue, or one 5 to 10 µm thick formalin-fixed, paraffin-embedded tissue section. 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.

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

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 (Vial 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. All other reagents remaining in the Reagent Tubs after completion of the run may be used for the next run if performed on 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 II (Tissue), two different purification protocols are available. The protocol names listed below should appear on the 'Ordering' sub-tab of the MagNA Pure LC 2.0 Instrument.

Protocol Name	Sample Material	Procedure
DNA II Tissue	1 to 10 mg fresh-frozen tissue samples	▪ Transfer of homogenized sample to the MagNA Pure LC Reagent/Sample Stage. ▪ DNA purification is performed automatically by the Instrument. ▪ Sample volume: 80 µl to 90 µl ▪ Elution volume: 200 µl
DNA II Tissue External_ Proteinase K	5 to 10 µm deparaffinized section of formalin-fixed, paraffin-embedded tissue, or tissues that are difficult to homogenize.	▪ Proteinase K digestion is performed outside the Instrument. ▪ Transfer of lysate to the Reagent/Sample Stage. ▪ DNA purification is performed automatically by the Instrument. ▪ Sample volume: 100 µl to 110 µl ▪ Elution volume: 200 µl

All "DNA II Tissue" purification protocols enable the eluate to be diluted with up to 900 µl Elution Buffer.

Pre-Isolation Steps for Fresh-Frozen Tissue

Efficient disruption and homogenization of the sample material is essential for the isolation of genomic DNA from tissues. Incomplete tissue disruption will result in significantly reduced DNA yields. Excessive disruption and homogenization will lead to shearing of high-molecular weight genomic DNA. For best results, do not exceed the recommended times. With some disruption methods, the sample is simultaneously lysed and homogenized, while others require an additional homogenization step.

⚠ ***Always freshly prepare tissue lysates and process them immediately. If necessary, store the lysate at -15 to -25°C.***

Tissue Disruption Using the MagNA Lyser Instrument

This procedure describes the disruption and homogenization of fresh-frozen, or RNA*later*-fixed tissue using the MagNA Lyser Instrument*.

1. Add 80 µl Tissue Lysis Buffer (Bottle 7) to a MagNA Lyser Green Beads* tube.

2. Transfer 1 to 10 mg tissue into the tube.

   Start with a minimum of double the amount of tissue and buffer for homogenization since part of the sample lysate (bound between the homogenization beads) cannot be utilized.

3. Set up the MagNA Lyser Instrument as described in the Operator's Manual.

4 Start the disruption cycle, applying speed and time settings appropriate for the specific sample material.

⚠ ***Always optimize the tissue disruption parameters (speed, time) prior to performing the actual DNA purification procedure. Insufficient disruption may lead to poor DNA yields, while excessive disruption may lead to DNA shearing.***

- Refer to the following table for values of exemplary sample materials:

Sample Material	Speed	Time [s]
Liver/Kidney	6,500 rpm	15 - 20
Spleen/Tumor Tissue RNA*later*-fixed Tissue		>25[(1)]
Tail/Ear/Skin		>25[(1)]

5 Centrifuge 2 minutes at 13,000 × *g* at +15 to +25°C.

6 Transfer 80 µl of the lysate supernatant into the Sample Cartridge.

7 Place the Sample Cartridge on the Reagent/Sample Stage and start the "DNA II Tissue" protocol, as described in section **DNA Isolation Protocol**.

(1) Long disruption cycles may cause degradation of DNA by heat stress. Avoid continuous disruption of cycles of more than 25 seconds. Instead, apply several short disruption cycles. Cool the samples in the MagNA Lyser Rotor Cooling Block supplied with the MagNA Lyser Instrument, or on ice between the disruption cycles.

Tissue Disruption Using a Rotor-Stator Homogenizer

This procedure describes the disruption and homogenization of fresh-frozen, or RNA*later*-fixed tissue using a rotor-stator homogenizer, such as Ultra Turrax or Omni TH 220.

1 Lyse and homogenize tissue with 80 µl Tissue Lysis Buffer (Bottle 7) in a rotor-stator homogenizer, following instrument supplier instructions. Depending on the type of sample, this takes approximately 5 to 30 seconds.

ⓘ *Depending on the type of tissue, several disruption cycles may be necessary.*

⚠ ***Always hold and keep the tip of the homogenizer submerged and to one side of the tube to avoid the development of foam.***

2 Centrifuge 2 minutes at 13,000 × *g* at +15 to +25°C.

3 Transfer 80 µl of the lysate supernatant into the Sample Cartridge.

4 Place the Sample Cartridge on the Reagent/Sample Stage and start the "DNA II Tissue" protocol, as described in section **DNA Isolation Protocol**.

Tissue Disruption Using Mortar/Pestle/Syringe

This procedure describes disruption and homogenization of fresh-frozen, or RNA*later*-fixed tissue using a mortar, pestle, and syringe.

1. Thoroughly grind 1 to 10 mg tissue in liquid nitrogen with a mortar and pestle.
   - Transfer the frozen powder into a liquid nitrogen pre-cooled microfuge tube suitable for centrifugation.
   - Allow the remaining liquid nitrogen to evaporate, but avoid thawing of the tissue sample.

2. Add 80 µl Tissue Lysis Buffer (Bottle 7) to the sample, then homogenize further by passing the sample through a 0.8 mm syringe needle 6 times.

3. Centrifuge 2 minutes at 13,000 × *g* at +15 to +25°C.

4. Transfer 80 µl of the lysate supernatant into the Sample Cartridge.

5. Place the Sample Cartridge on the Reagent/Sample Stage and start the "DNA II Tissue" protocol, as described in section DNA Isolation Protocol.

RNA Digestion Step (Optional)

Some tissue types may contain large amounts of RNA. To eliminate RNA co-isolated from tissues, add 10 µl of an RNase A* solution (40 mg/ml, dissolved in Elution Buffer) to 80 µl lysate and incubate for 15 minutes at +65°C.

i *This step may increase DNA yield.*

External Proteinase K Digestion Step (Optional)

To facilitate the homogenization of difficult sample materials, such as certain types of tumor tissue, use the "DNA II Tissue External_Proteinase K" purification protocol. This protocol enables the performance of an extensive Proteinase K digestion prior to automated DNA isolation.

1. Add 20 µl of Proteinase K solution (see section **Working Solution**) to the sample lysate.
   - Incubate at +55 to +65°C 30 minutes to overnight or until digestion is finished.

2. Centrifuge samples briefly and transfer the supernatant to the Sample Cartridge.

3. Place the Sample Cartridge on the Reagent/Sample Stage and start the "DNA II Tissue External_Proteinase K" protocol, as described in section **DNA Isolation Protocol**.

Pre-Isolation Steps for Formalin-Fixed, Paraffin-Embedded Tissue Sections

The MagNA Pure LC DNA Isolation Kit II (Tissue) is optimized for the isolation of DNA from paraffin-embedded tissue sections fixed with 10% neutral buffered formalin. Other tissue fixation procedures may affect the DNA yield.

Deparaffinization on a Microscope Slide

This procedure describes the deparaffinization procedure for one section of formalin-fixed, paraffin-embedded tissue (5 to 10 µm thickness) directly on a microscope slide.

1. Place the slide in a Hemo-De or xylol bath.
2. Incubate for 10 minutes.
3. Tap off excess liquid and place the slide in absolute ethanol for 10 minutes.
4. Change the bath or transfer the slide into fresh absolute ethanol for another 10 minutes.
5. Scratch the deparaffinized section from the slide using a sterile, single-use scalpel and place the section into a 1.5 ml reaction tube.
   To avoid tissue dispersing and loss of sample, scratch the section from the microscope slide before it has dried.
6. Dry the tissue pellet for 10 minutes at +55°C.
7. Add 80 µl Tissue Lysis Buffer (Bottle 7) and 20 µl Proteinase K solution (see section **Working Solution**) to the deparaffinized dry tissue pellet.
8. Vortex briefly several times.
9. Incubate overnight at +55°C with continuous shaking.
   Additional mixing by pipetting up and down after 1 hour of incubation may facilitate homogenization, avoiding an additional Proteinase K digestion step.
10. Centrifuge for 1 minute at 8,000 rpm.
11. If there is a visible pellet, add an additional 10 µl Proteinase K solution (see section **Working Solution**).
12. Vortex briefly several times and incubate for one hour at +55°C.
13. Transfer the tissue lysate into the Sample Cartridge.
14. Place the Sample Cartridge on the Reagent/Sample Stage and start the “DNA II Tissue External_Proteinase K” protocol, as described in section **DNA Isolation Protocol**.

Deparaffinization Within a Reaction Tube

This procedure describes the deparaffinization procedure for one section of formalin-fixed, paraffin-embedded tissue (5 to 10 µm thickness) in a 1.5 ml reaction tube.

1. Add 800 µl Hemo-De or xylol to the tissue section in a 1.5 ml reaction tube.
   - Invert several times to mix.

2. Add 400 µl absolute ethanol to the Hemo-De or xylol and mix gently by inverting the tube several times.

3. Centrifuge for 2 minutes at maximum speed (12,000 to 14,000 rpm), then discard the supernatant.

4. Add 1 ml absolute ethanol and mix gently by inverting the tube several times.

5. Centrifuge for 2 minutes at maximum speed and discard the supernatant.

6. Blot the tube briefly on paper towel to remove residual ethanol.

7. Dry the tissue pellet for 10 minutes at +55°C.

8. Add 80 µl Tissue Lysis Buffer (Bottle 7) and 20 µl Proteinase K solution (see section **Working Solution**) to the deparaffinized dry tissue pellet.

9. Vortex briefly several times.
   - Incubate overnight at +55°C with continuous shaking.

   Additional mixing by pipetting up and down after 1 hour incubation may facilitate homogenization of the tissue, avoiding an additional Proteinase K digestion step after the overnight incubation.

10. Centrifuge for 1 minute at 8,000 rpm.

11. If there is a visible pellet, add an additional 10 µl Proteinase K solution (see section **Working Solution**).

12. Vortex briefly several times and incubate for one additional hour at +55°C.

13. Transfer the tissue lysate into the Sample Cartridge.

14. Place the Sample Cartridge on the Reagent/Sample Stage and start the “DNA II Tissue External_Proteinase K” protocol, as described in section **DNA Isolation Protocol**.

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 section **'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 or nuclease-free 10 mM Tris-HCl, pH 8.0 as dilution buffer.

Isolate genomic DNA according to the following protocol:

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.....	Then use the....
unlysed fresh-frozen tissue samples	"DNA II Tissue" protocol
externally lysed tissue samples	"DNA II Tissue External_Proteinase K" protocol

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 (stable for at least several weeks).
- For long-term storage, it is recommended to 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, 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 can 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

Integrity

DNA was purified from different types of tissue and analyzed on agarose gels. The DNA was of high quality with an average molecular weight of approximately 10 kb and showed no signs of degradation (Fig. 1). DNA from formalin-fixed, paraffin-embedded tissue may be of lower integrity, depending on age and tissue type, fixative, and the fixation protocol used.

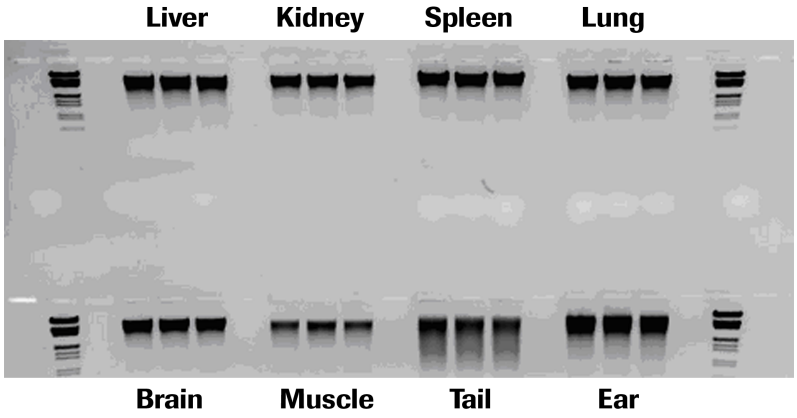

Fig. 1: Genomic DNA was isolated from different types of fresh-frozen mouse tissue in triplicates, and analyzed on a 1% agarose gel. The bands illustrate DNA of high integrity.

Yield

DNA was prepared from different amounts and types of tissues. Yields were determined by OD_{260} measurement and gel analysis, and compared to conventional methods. DNA yields were equal to or higher than those from filter tube methods. Quantitative PCR analysis using the LightCycler® Carousel-Based System confirmed these data, see **Typical Results Table**.

Purity

DNA was purified from different amounts and types of tissue sample materials. Typical experiments produced an $OD_{260/280}$ ratio of 1.8 ± 0.1, indicating highly purified DNA. No inhibition was observed in PCR analysis using the LightCycler® Carousel-Based System, see **Typical Results Table**.

Typical Results From Fresh-Frozen Mouse Tissue

Tissue Type	Amount [mg]	DNA Yield [µg]	Purity [$OD_{260/280}$]	CP[1]
Liver	10	18	1.8	18.6
Kidney	10	18	1.8	18.7
Tail	10	10	1.7	19.7
Spleen	10	40	1.9	18.0
Brain	10	22	1.7	18.1
Ear	10	16	1.7	19.9
Muscle	10	4	1.7	20.9
Lung	10	25	1.8	18.0

[1] CP = Crossing points reflect the isolated amount of DNA and are calculated after PCR analysis using the LightCycler® Carousel-Based System. Quantification is based on using a DNA standard.

Scalability

DNA was isolated from different amounts of tissue (1, 2, 5, and 10 mg of mouse liver) and the results were examined by OD_{260} measurement, (Fig. 2), agarose gel electrophoresis (Fig. 3), and PCR analysis using the LightCycler® Carousel-Based System (Fig. 4). All data reflected good scalability.

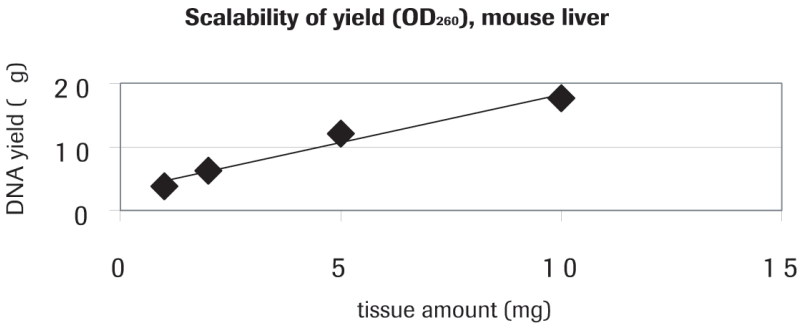

Fig. 2: Scalability of DNA yield from fresh-frozen mouse liver as determined by OD_{260} measurement.

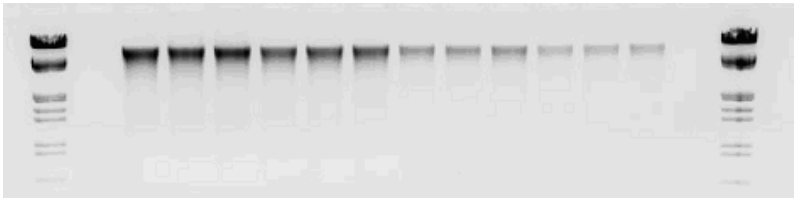

Fig. 3: Scalability of DNA yield from fresh-frozen mouse liver, as determined by agarose gel analysis.

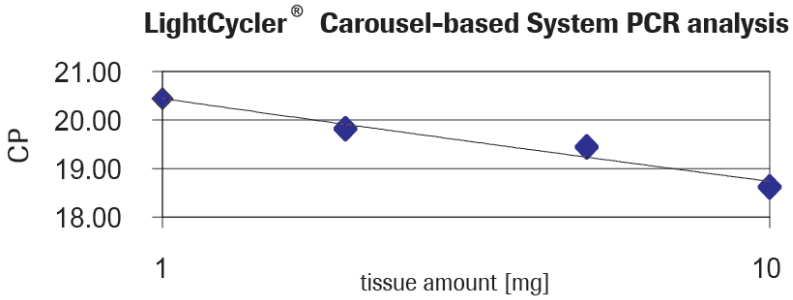

Fig. 4: Scalability of DNA yield from fresh-frozen mouse liver as determined by PCR analysis using the LightCycler® Carousel-Based System.

Reproducibility

Eighteen samples of 10 mg mouse liver were subjected to the DNA isolation protocol. Agarose gel electrophoresis (Fig. 5), OD measurement, and PCR analysis using the LightCycler® Carousel-Based System showed good reproducibility. The coefficient of variance (CV) of DNA yield and purity were <10%, and the CV for the crossing points was <3%.

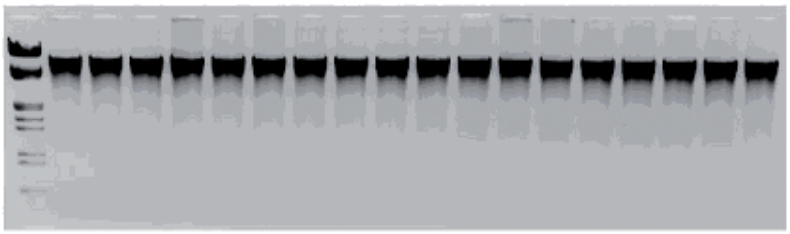

Fig. 5: Reproducibility: DNA isolation from 18 samples of 10 mg fresh-frozen mouse liver.

Relative Quantification of DNA Isolation From Formalin-Fixed, Paraffin-Embedded Breast Tumor Tissue Sections

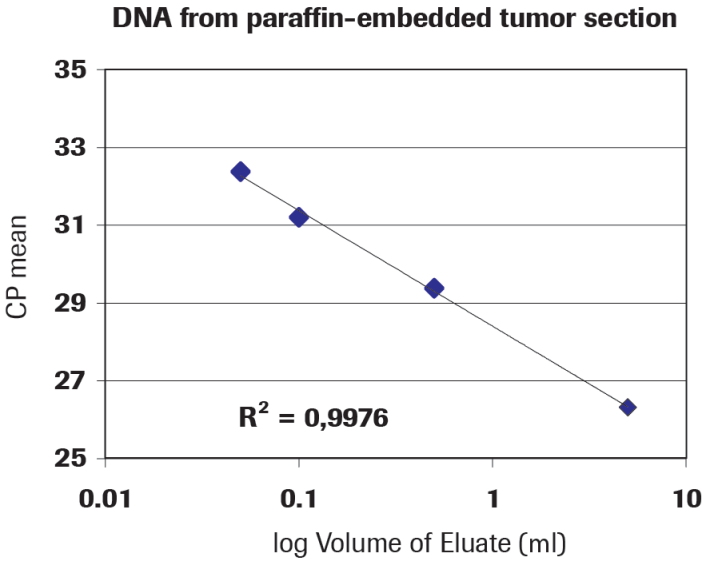

Fig. 6: A serial dilution of DNA isolated from a formalin-fixed, paraffin-embedded section which was amplified with the LightCycler® HER2/neu DNA Quantification Kit. The relative ratio of the HER2/neu signal to the reference gene was linear in the range of the two log stages tested.

Downstream Applications

DNA from different types of mouse tissue was tested in PCR analysis using the LightCycler® Carousel-Based System, blockcycler PCRs, restriction digest, and Southern blots. The DNA was suitable for all experiments and showed identical or improved performance compared to DNA prepared with conventional methods.

4. Troubleshooting

Observation	Possible cause	Recommendation
Clumping of beads or presence of beads in Storage Cartridge.	Too much sample material or inefficient homogenization method.	Reduce amount of sample material to the values indicated in the section **Sample Material**.
		Use recommended homogenization methods, see section **Pre-Isolation Steps for Fresh-Frozen Tissue**.
	MGPs were magnetized prior to use.	Avoid contact between MGPs and magnets prior to use.
		Store kit properly.
DNA is degraded.	Samples stored incorrectly.	Use fresh or frozen samples.
		Avoid the use of samples that have been stored extensively at +15 to +25°C.
	Homogenization too intensive.	Shorten homogenization.
Poor DNA yield.	Sample did not contain enough material.	Weigh tissue before use. *Optimal results are obtained using 1 to 10 mg tissue. For some tissue types, up to 25 mg may be used.*
	Too much sample material.	Reduce amount of sample material to the values indicated in the section **Sample Material**.
	Inefficient homogenization.	Use recommended homogenization methods, see section **Pre-Isolation Steps for Fresh-Frozen Tissue**.
		Cut tissue into small pieces prior to homogenization, and use more intensive homogenization.
	Samples stored incorrectly.	Use fresh or frozen samples.
		Avoid the use of samples that have been stored extensively at +15 to +25°C.
Poor DNA purity.	Too much sample material.	Reduce amount of sample material to the values indicated in the section **Sample Material**.

Poor PCR performance.	Too much DNA in PCR.	Check DNA concentration of eluates and adjust amount of eluate per PCR, or dilute the eluate, for example, 1 in 10. *Optimal: 1 to 100 ng/PCR maximum: approximately 1 µg.*
		Use less starting material for DNA isolation. *Optimal results are obtained with 1 to 10 mg tissue per isolation.*
	Not enough DNA in PCR.	Check DNA concentration of eluates and adjust amount of eluate per PCR. *Optimal: 1 to 100 ng/PCR maximum: approximately 1 µg.*
		Use more starting material.
		Centrifuge to concentrate sample.
	Poor purity of DNA.	Use less sample material for DNA isolation. *Optimal results are obtained with 1 to 10 mg tissue per isolation.*
	PCR reagents and protocol were not optimal.	Check PCR reagents and protocols with a positive DNA control, such as 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.
	No PCR products from DNA of formalin-fixed, paraffin-embedded tissue.	Check PCR reagents and protocols with a positive DNA control, such as Human Genomic DNA*.
		If there is an appropriate PCR product for a control, design a smaller PCR amplicon for amplification of formalin fixed, paraffin-embedded tissue (e.g., 100 to 400 bp).
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 using LightCycler® Instruments.*

5. Additional Information on this Product

5.1. Test Principle

The tissue samples or paraffin sections are first homogenized using the MagNA Lyser Instrument or other suitable homogenization device, using the Tissue Lysis Buffer supplied in the kit. Homogenization of formalin-fixed, paraffin-embedded tissue sections is performed by an overnight incubation with Proteinase K. Sample lysates are then transferred to the MagNA Pure LC Instrument. The isolation procedure is based on magnetic-bead technology. Samples are further 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. 7).

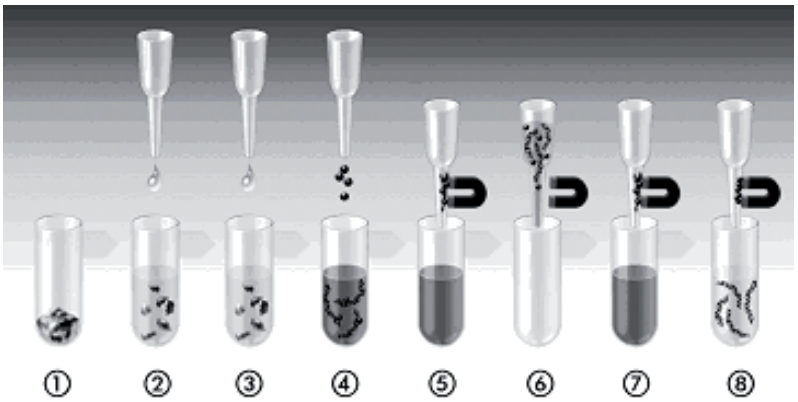

Fig. 7: Principle of the MagNA Pure LC DNA Isolation Kit II (Tissue).The principle steps of a MagNA Pure LC DNA isolation procedure are:

① Sample lysate 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, such as 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

DNA II Tissue Protocol

The basic steps of the protocol are:

① Dispense Proteinase K to samples into the Sample Cartridge, mix, and incubate.

② Dispense all required reagents into the Processing Cartridge.

③ Add Lysis/Binding Buffer to the sample and mix.

④ Transfer lysate into MGP suspension, 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, mix, incubate, and elute DNA.
- Discard MGPs.

⑨ Transfer eluate to the Storage Cartridge (Cooling Unit I).

5.2. Quality Control

DNA is isolated from mouse tissue using the standard protocol and analyzed with respect to integrity, yield, purity, and ability to amplify in 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

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)		
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, 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, clear	5 x 10 plates	05 102 413 001
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® 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
Reagents, kits		
MagNA Lyser Green Beads	100 tubes, prefilled with ceramic beads	03 358 941 001
Human Genomic DNA	100 µg, 500 µl	11 691 112 001
RNase A	25 mg	10 109 142 001
	100 mg	10 109 169 001

6.4. Trademarks

MAGNA LYSER, 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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