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

**Cat. No. 03 186 229 001**

192 isolations from fresh-frozen tissue or formalin-fixed tissue

Store the kit at +15 to +25°C
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1. What this Product Does

Number of Tests
• 192 isolations from up to 1 to 10 mg fresh-frozen tissue or one 5 to 10 μm formalin-fixed/paraffin-embedded tissue section.

The kit is designed to process up to 192 samples in batches of 32. When processing fewer than 32 samples at a time, some reagent will be wasted and the remaining reagent will not be enough to process 192 samples.

Kit Contents

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

<table>
<thead>
<tr>
<th>Bottle/Cap Label</th>
<th>Contents / Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>black 1</td>
<td>Wash Buffer I</td>
</tr>
<tr>
<td>blue 2</td>
<td>Wash Buffer II</td>
</tr>
<tr>
<td>red 3</td>
<td>Wash Buffer III</td>
</tr>
<tr>
<td>green 4</td>
<td>Lysis/Binding Buffer</td>
</tr>
<tr>
<td>caramel 5</td>
<td>Magnetic Glass Particles (MGPs) Suspension</td>
</tr>
<tr>
<td>yellow 6</td>
<td>Elution Buffer</td>
</tr>
<tr>
<td>clear T</td>
<td>Tissue Lysis Buffer</td>
</tr>
<tr>
<td>pink P</td>
<td>Proteinase K</td>
</tr>
</tbody>
</table>

- 2 bottles, 100 ml each
- for removal of PCR inhibitors
- 2 bottles, 64 ml each
- for removal of salts, proteins etc.
- 1 bottle, 100 ml
- for removal of residual salts
- 1 bottle, 100 ml
- for cell lysis and binding of DNA
- 6 glass vials, MGP suspension
- for binding of DNA
- 1 bottle, 100 ml
- for elution of DNA
- for reconstitution of Proteinase K
- for dilution of eluates (optional)
- 1 bottle, 100 ml
- for efficient lysis of tissue
- 6 glass vials, lyophilizate
- for digestion of proteins

Storage and Stability
- Kit components are stable at +15 to +25°C until the expiration date printed on the label.

The kit is shipped at ambient temperture.
**Additional Equipment and Reagents Required**

- standard laboratory equipment
- pipettes and nuclease-free, aerosol-preventive tips, to predisperse samples into the MagNA Pure LC Sample Cartridge
- centrifuge and suitable nuclease-free reaction tubes
- vortex mixer, to resuspend the MGPs
- heating device (65°C/95°C, for reaction tubes: 1.5 to 2 ml)
- homogenization device:
  - MagNA Lyser Instrument* with MagNA Lyser Green Beads*
  - rotor-stator homogenizer (e.g., UltraTurrax or Omni TH 220)
  - mortar/pestle/needle (Ø 0.8 mm)
- Hemo-De or Xylol
- Ethanol, absolute

*available from Roche Applied Science; see Ordering Information for details.

**Application**

For general laboratory use. The MagNA Pure LC DNA Isolation Kit II (Tissue), a General Purposes Reagent (GPR), is designed for use with the MagNA Pure LC Instruments [MagNA Pure LC 1.0 Instrument (Cat. No. 12 236 931 001) and MagNA Pure LC 2.0 Instrument (Cat. No. 05 197 686 001)], 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.

**Assay Time**

Set-up of the MagNA Pure LC Instruments requires approximately 15 min. Total time for the automated purification of DNA from 32 samples is approximately:

- 100 min, for fresh-frozen tissue samples
- 60 min, for deparaffinized sections of formalin-fixed/paraffin-embedded and difficult to-homogenize tissue samples (which require an external Proteinase K digestion).

No hands-on time is required after set up of MagNA Pure LC Instruments. Extra hands-on time is required for the manual pre-isolation steps.
2. How to Use This Product

2.1 Before You Begin

Precautions

I) Handling Requirements
- Complete each phase of the PCR workflow before proceeding to the next phase. For example, you should finish PCR sample preparation before starting PCR set-up. Sample preparation, PCR set up, and the PCR run should be performed in separate locations.
- Do not pool reagents from different lots or from different bottles of the same lot.
- Do not use a kit after its expiration date has passed.
- Wash Buffer I (bottle 1), Lysis/Binding Buffer (bottle 4) and Tissue Lysis Buffer (bottle T) contain guanidinium salts, which are irritants. Do not let Wash Buffer I, Lysis/Binding Buffer or Tissue Lysis Buffer touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If you spill the reagent, dilute the spill with water before wiping it up.
- Do not allow the Lysis/Binding Buffer to mix with sodium hypochlorite (bleach) solution or strong acids. This mixture can produce a highly toxic gas.

II) Laboratory Procedures
- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis/Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease-free pipet tips only, to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.

III) Waste Handling
- Discard unused reagents and waste in accordance with country, federal, state and local regulations.
- Material Safety Data Sheets (MSDS) are available on the Roche Applied Science home page (http://www.roche-applied-science.com), or upon request from the local Roche office.
To perform DNA isolations using MagNA Pure LC 1.0 Instruments with the MagNA Pure LC DNA Isolation Kit II (Tissue), new purification protocols for the MagNA Pure LC Software, Version 2.11 (or lower) must be installed. If not previously installed, order the protocols free of charge. If running Software Version 3.0 or above, or MagNA Pure LC 2.0 Software, no extra protocol installation is required. For additional details, contact your Roche representative.

To perform DNA isolations on all MagNA Pure LC Instruments with the MagNA Pure LC DNA Isolation Kit II (Tissue), two different purification protocols are available. The protocol names, listed below, should appear in the protocol selection of the “Sample Ordering” screen of the MagNA Pure LC 1.0 Instrument, or on the “Ordering” sub-tab of the MagNA Pure LC 2.0 Instrument.

Use the following table to determine which protocol is best for your sample material or application.

<table>
<thead>
<tr>
<th>Protocol Name</th>
<th>Sample Material</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA II Tissue</td>
<td>1 to 10 mg fresh-frozen tissue samples</td>
<td>• the homogenized sample is transferred to the MagNA Pure LC Reagent/Sample Stage. DNA purification is performed automatically by the Instrument.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Sample volume: 80 µl to 90 µl</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Elution volume: 200 µl</td>
</tr>
<tr>
<td>DNA II Tissue</td>
<td>5 to 10 µm deparaffinized section of formalin-fixed/paraffin-embedded or difficult-to-homogenize tissues</td>
<td>• a Proteinase K digestion takes place outside the Instrument.</td>
</tr>
<tr>
<td>External_Proteinase K</td>
<td></td>
<td>• the lysate is then transferred to the Reagent/Sample Stage. DNA purification is performed automatically by the Instrument.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Sample volume: 100 µl to 110 µl</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Elution volume: 200 µl</td>
</tr>
</tbody>
</table>

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

Sample Material

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.

The optimal amount of sample material is, as follows:
• 1 to 10 mg fresh-frozen or unfrozen, stabilized (e.g., treated with RNAlater) tissue (depending on the tissue type)
• One formalin-fixed/paraffin-embedded tissue section (5 to 10 μm thick)
⚠️ Do not use more sample material than this kit and the protocol chosen is designed to handle. Doing so may affect the performance of the isolation process and may lead to clumping and loss of MGPs, or cross-contamination of samples.
⚠️ Treat all samples as potentially infectious.

2.2 Preparation of Working Solutions

Before starting the procedure, prepare the working solutions as described below.

👍 All other solutions are ready-to-use.
⚠️ All buffers are clear. Do not use a buffer, if it contains a precipitate. If a precipitate is visible, place the bottle at +37°C and mix from time to time until the precipitate is completely dissolved. Do not warm the buffer longer at +37°C than is actually needed to completely dissolve of the precipitate. Before using the buffer, equilibrate at room temperature.
⚠️ Equilibrate buffers and working solutions to room temperature before use. If you use the reagents at temperatures outside the recommended range, the kit may not function properly.
⚠️ Use only the reagent amount required for your number of samples.
⚠️ Do not store the Proteinase K or the MGP suspension in a Reagent 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.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Preparation/Comments</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnetic Glass Particles</td>
<td>The MGP suspension (vial 5) must be mixed thoroughly. Vortex immediately before use to produce a homogeneous suspension. The beads tend to sediment during storage. For best results, add the MGPs to the Instrument just before starting the run (to minimize sedimentation). Always use the exact amount of MGPs recommended by the software.</td>
<td>• Store MGPs at +15 to +25°C. Do not store the MGP suspension in a Reagent Tub, or similar. Do not leave the MGP suspension uncovered in the bottle or in the Reagent Tub, as evaporation may lead to suboptimal purification.</td>
</tr>
</tbody>
</table>
Controls

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. In order to control the complete process starting from sample preparation to quantification analysis, perform the following controls:

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

⚠️ 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.

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.

### Reagent | Preparation/Comments | Storage
--- | --- | ---
Proteinase K | Reconstitute each vial of Proteinase K (vial P) 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. | 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.

⚠️ One vial of Proteinase K is sufficient for 32 samples.

For quantification assays on the LightCycler® Instruments, use a synthetic double-stranded DNA molecule with primer-binding sites identical to those of your target sequence, but having a unique probe-binding region, that differentiates the IC from the target-specific amplicon. Discriminate the signals derived from your target and the IC, by performing a dual-color HybProbe Assay. For detailed information, regarding the IC concept, in combination with the LightCycler® Carousel-Based System, read the LightCycler® Technical Note 12/2000 “Absolute Quantification with External Standards and an Internal Control”, available at http://www.lightcycler-online.com.
2.3 Pre-Isolation Steps for Fresh-Frozen Tissue

General Remarks
- Efficient disruption and homogenization of the sample material is essential for isolation of genomic DNA from tissues. Incomplete tissue disruption will result in significantly reduced DNA yields. On the other hand, too extensive disruption and homogenization, will lead to shearing of high-molecular weight genomic DNA. For best results, do not exceed the recommended times, listed below.
- In some disruption methods, the sample is simultaneously lysed and homogenized, while in other disruption methods, an additional homogenization step is required.

⚠️ Always freshly prepare tissue lysates and process them immediately. Store the lysate at −15 to −20°C or below when DNA isolation is to be postponed.

Tissue Disruption

Using the MagNA Lyser Instrument

The table below describes disruption and homogenization of fresh-frozen, or RNAlater-fixed tissue, using the MagNA Lyser Instrument*.

1. Add 80 μl Tissue Lysis Buffer (bottle T) to a MagNA Lyser Green Beads* tube. Then transfer 1 to 10 mg tissue into the tube.

⚠️ It is beneficial to start with (at least) double the amount of tissue and buffer for homogenization, because part of the sample lysate (bound between the homogenization beads) can not be utilized.

- Set up the MagNA Lyser Instrument, as described in the Operator's Manual.
- 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 yield, while excessive disruption may lead to DNA shearing. As an initial starting point, use the values given for some exemplary sample materials in the table below:

<table>
<thead>
<tr>
<th>Sample Material</th>
<th>Speed</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver/Kidney</td>
<td>6,500 rpm</td>
<td>15 to 20 s</td>
</tr>
<tr>
<td>Spleen/Tumor Tissue/Tail/Ear/Skin RNAlater-fixed Tissue</td>
<td>6,500 rpm</td>
<td>&gt;25 s*</td>
</tr>
</tbody>
</table>

⚠️ Long disruption cycles may cause degradation of DNA by heat stress. Although DNA tends to be more resistant against heat stress than against mechanical stress, avoid continuous disruption cycles of more than 25 s. Rather, apply several short disruption cycles. Cool the samples in the MagNA Lyser Rotor Cooling Block* (included with the MagNA Lyser Instrument), or on ice, between the disruption cycles.

2. Centrifuge 2 min 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 2.5.
Tissue Disruption Using a Rotor-Stator Homogenizer

The table below describes disruption and homogenization of fresh-frozen, or RNA/later-fixed tissue, using a rotor-stator homogenizer (e.g., UltraTurrax or Omni TH 220).

1. Lyse and homogenize tissue with 80 μl Tissue Lysis Buffer (bottle T) in a rotor-stator homogenizer, following instrument supplier instructions. Depending on the type of sample, rotor-stator homogenizers thoroughly disrupt and homogenize tissue in 5 to 30 s, in the presence of Tissue Lysis Buffer.
   - Depending on the type of tissue, several disruption cycles may be necessary.
   - Avoid generating of foam by keeping the tip of the homogenizer always submerged and holding the immersed tip to one side of the tube.

2. Centrifuge 2 min 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 2.5.

Tissue Disruption Using Mortar/ Pestle/Syringe

The table below 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, that is suitable for centrifugation.
   - Allow the remaining liquid nitrogen to evaporate, but avoid thawing of the tissue sample.
   - Add 80 μl Tissue Lysis Buffer (bottle T) to the sample, then homogenize further by passing the sample through a syringe needle (∅ 0.8 mm) 6 times.

2. Centrifuge 2 min 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 2.5.
Optional: RNA Digestion Step

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

This step may increase DNA yield.

Optional: External Proteinase K Digestion Step

To facilitate the homogenization of difficult sample materials (e.g., certain types of tumor tissue), the “DNA II Tissue External Proteinase K” purification protocol is available. This protocol enables the performance of an extensive Proteinase K digestion, prior to automated DNA isolation:

- Add 20 μl of Proteinase K solution (prepared in section 2.2) to the sample lysate and incubate at +55 to +65°C, until digestion is complete (30 min to overnight).
- Centrifuge samples briefly and transfer the supernatant to the Sample Cartridge. Place the Sample Cartridge on the Reagent/Sample Stage and start the “DNA II Tissue External Proteinase K” protocol, as described in section 2.5.

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

General Remarks

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

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

<table>
<thead>
<tr>
<th>Step</th>
<th>Instruction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Place the slide in a Hemo-De (or Xylol) bath, then incubate for 10 min.</td>
</tr>
<tr>
<td>2</td>
<td>Tap off excess liquid, then place the slide into absolute ethanol for 10 min.</td>
</tr>
<tr>
<td>3</td>
<td>Change the bath, then incubate the slide for an additional 10 min in absolute ethanol.</td>
</tr>
<tr>
<td>4</td>
<td>Scratch the deparaffinized section from the slide, using a sterile, single-use scalpel, then place the section into a 1.5 ml reaction tube.</td>
</tr>
<tr>
<td>5</td>
<td>Dry the tissue pellet for 10 min at +55°C.</td>
</tr>
</tbody>
</table>

To avoid dispersing of the tissue and loss of sample, scratch the section from the microscope slide before it has dried.
### Deparaffinization within a Reaction Tube

<table>
<thead>
<tr>
<th>Step</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Add 800 μl Hemo-De (or Xylol) to one 5 to 10 μm tissue section in a 1.5 ml reaction tube. Invert several times to mix.</td>
</tr>
<tr>
<td>2</td>
<td>Add 400 μl absolute ethanol to the Hemo-De (or Xylol) and mix gently by inverting the tube several times.</td>
</tr>
<tr>
<td>3</td>
<td>Centrifuge for 2 min at maximum speed (12,000 to 14,000 rpm), then discard the supernatant.</td>
</tr>
<tr>
<td>4</td>
<td>Add 1 ml absolute ethanol and mix gently by inverting the tube several times.</td>
</tr>
</tbody>
</table>
| 5    | • Centrifuge for 2 min at maximum speed and discard the supernatant.  
• Blot the tube briefly on paper towel to remove residual ethanol. |
| 6    | Dry the tissue pellet for 10 min at +55°C. |
| 7    | • Add 80 μl Tissue Lysis Buffer (bottle T) and 20 μl Proteinase K solution (prepared in section 2.2) to the deparaffinized dry tissue pellet.  
• Vortex briefly several times, then 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. |

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

<table>
<thead>
<tr>
<th>Step</th>
<th>Instructions</th>
</tr>
</thead>
</table>
| 8    | Check whether lysis is complete, by centrifugation for 1 min at 8,000 rpm.  
• If there is a visible pellet, then add an additional 10 μl Proteinase K solution (prepared in section 2.2).  
• Vortex briefly several times, then incubate for one additional hour at +55°C. |
| 9    | Transfer the tissue lysate into the Sample Cartridge.  
Place the Sample Cartridge on the Reagent/Sample Stage and start the “DNA II Tissue External_Proteinase K” protocol, as described in section 2.5. |
2.5 DNA Isolation Protocol

General Remarks

- The following procedure is designed to process 32 samples at the same time. If you are processing fewer samples, the software will reduce the volumes of all solutions accordingly (see the ‘Start Information’ screen of the MagNA Pure LC 1.0 Instrument, or the ‘Stage Setup’ sub-tab of the MagNA Pure LC 2.0 Instrument).
- The software automatically calculates the necessary amounts of reagents and disposable plastics and guides you through the set-up.
- You can not start the Instrument unless the Disposable Lockbar, for securing the Sample Cartridge, Reagent Tubs and Reaction Tips, is closed.
- If you programmed dilution of the eluate, you will need an additional Reagent Tub M30 in position R7. Use Elution Buffer or nuclease-free 10 mM Tris-HCl, pH 8.0 as dilution buffer.

Protocol

Isolate genomic DNA according to the protocol below.

Start Instrument and Software

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Pure LC 1.0</td>
<td>Turn on the Instrument and the computer, then start the MagNA Pure LC 1.0 Software. Navigate to the ‘Start Information’ screen.</td>
</tr>
<tr>
<td>MagNA Pure LC 2.0</td>
<td>Turn on the Instrument, the MagNA Pure LC 2.0 Software starts automatically. Log in and then navigate to the ‘Ordering’ sub-tab.</td>
</tr>
</tbody>
</table>
### All MagNA Pure LC Instruments

- Select the appropriate protocol:
  - **If you are starting with.....**
  - unlysed fresh-frozen tissue samples
  - externally lysed tissue samples

- Then use....
  - the “DNA II Tissue” protocol
  - the “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

<table>
<thead>
<tr>
<th>All MagNA Pure LC Instruments</th>
<th>Before starting the isolation procedure, fill all Reagent Tubs outside the Instrument with the required amount of reagents equilibrated to room temperature.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Pure LC 1.0 Instrument</td>
<td>Fill each Reagent Tub with the volume listed on the ‘Start Information’ screen, then close it with a Tub Lid.</td>
</tr>
<tr>
<td>MagNA Pure LC 2.0 Instrument</td>
<td>Fill each Reagent Tub with the volume listed on the ‘Stage Setup’ sub-tab, then close it with a Tub Lid.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>All MagNA Pure LC Instruments</th>
<th>□ Close Reagent Tubs with the Tub Lids to prevent evaporation of the reagents. However, even when closed, Reagent Tubs are not suitable for long-term storage of reagents.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>□ Load the exact amount of MGPs (as listed on the ‘Start Information’ screen or ‘Stage Setup’ sub-tab) on to the Instrument just before the run starts. This will prevent them from sedimenting.</td>
</tr>
</tbody>
</table>

### Set Up Reagent Tubs and Disposables on the Reagent/Sample Stage

| MagNA Pure LC 1.0 Instrument  | Use the information of the ‘Start Information’ screen to place all disposable plastics and reagents within Reagent Tubs, necessary for the batch run on the Reagent/Sample Stage. |
| MagNA Pure LC 2.0 Instrument  | Use the information of the ‘Stage Setup’ sub-tab to place all disposable plastics and reagents within Reagent Tubs, necessary for the batch run on the Reagent/Sample Stage. |

| All MagNA Pure LC Instruments | ☀ A colored “Positioning Frame”* that can be placed on the Reagent Reservoir Rack to aid correct loading of the reagents, is available with the MagNA Pure LC Disposables Starter Set. |

*Color information is not applicable in text format.
Storage of DNA Eluates

To ensure greatest possible stability of the eluted nucleic acids, immediately proceed with PCR set-up. Do not store the eluted nucleic acid in the MagNA Pure LC Storage Cartridge on Cooling Unit 1.

For storage, close the Storage Cartridge 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. Ensure that the preparation is not repeatedly frozen and thawed before later analyses.

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*, standard PCR tubes or plates; see the MagNA Pure LC Operator’s Manuals for recommended plates. For Post Elution procedures, you can place LightCycler® Capillaries in the removable MagNA Pure LC Cooling Block, LC Centrifuge Adapters*, or the MagNA Pure LC Cooling Block, LC Sample Carousel*. Alternatively, you can place a LightCycler® 480 Multiwell Plate 96* into the MagNA Pure LC Cooling Block, 96-well PCR Plate*, in combination with the MagNA Pure LC 2.0 LightCycler® 480 Plate Adapter*. You can program the Post Elution steps either before you perform the isolation procedure, or after it is complete. See the MagNA Pure LC Operator’s Manuals, for details on how to set up a Post Elution run.
3. Results

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 approx. 10 kb and showed no signs of degradation (see 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 (see Ref. 2).

![Genomic DNA analysis on agarose gel](Image)

**Fig. 1:** Genomic DNA was isolated from different types of fresh-frozen mouse tissue (in triplicates, row 1: liver, kidney, spleen, lung; row 2: brain, muscle, tail, ear) and analyzed on a 1% agarose gel. The bands reveal DNA of high integrity.

Yield

DNA was prepared from different amounts and types of tissues. Yields were determined by OD260 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 Table below).

Purity

DNA was purified from different amounts and types of tissues. Typical experiments produced an OD260/280 ratio of 1.8 ± 0.1, which is indicative of high purity DNA. No inhibition was observed in PCR analysis, using the LightCycler® Carousel-Based System (see Table on next page).
Typical results from fresh-frozen mouse tissue

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Amount (mg)</th>
<th>DNA yield (μg)</th>
<th>Purity (OD_{260/280})</th>
<th>CP ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>10</td>
<td>18</td>
<td>1.8</td>
<td>18.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>10</td>
<td>18</td>
<td>1.8</td>
<td>18.7</td>
</tr>
<tr>
<td>Tail</td>
<td>10</td>
<td>10</td>
<td>1.7</td>
<td>19.7</td>
</tr>
<tr>
<td>Spleen</td>
<td>10</td>
<td>40</td>
<td>1.9</td>
<td>18.0</td>
</tr>
<tr>
<td>Brain</td>
<td>10</td>
<td>22</td>
<td>1.7</td>
<td>18.1</td>
</tr>
<tr>
<td>Ear</td>
<td>10</td>
<td>16</td>
<td>1.7</td>
<td>19.9</td>
</tr>
<tr>
<td>Muscle</td>
<td>10</td>
<td>4</td>
<td>1.7</td>
<td>20.9</td>
</tr>
<tr>
<td>Lung</td>
<td>10</td>
<td>25</td>
<td>1.8</td>
<td>18.0</td>
</tr>
</tbody>
</table>

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 (i.e., 1, 2, 5 and 10 mg of mouse liver) and the results were examined by OD_{260} (Fig. 2), agarose gel (Fig. 3) and PCR analysis using the LightCycler® Carousel-Based System (Fig. 4). All data reflected a good scalability.

**Scalability of yield (OD_{260}), mouse liver**

![Graph showing scalability of DNA yield from fresh-frozen mouse liver as determined by OD_{260} measurement.]

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

![Image showing agarose gel analysis.]

**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 (Fig. 5), OD 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 digests 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

<table>
<thead>
<tr>
<th>Possible Cause</th>
<th>Recommendation</th>
</tr>
</thead>
</table>
| **Clumping of beads or presence of beads in Storage Cartridge.** | • Reduce amount of sample material to the values indicated in the section “Sample Material”.  
• Use recommended homogenization methods (see section “2.3 Pre-Isolation Steps for Fresh-Frozen Tissue”).  
| MGPs were magnetized prior to use.                 | • Avoid contact between MGPs and magnets prior to use.                           
• Store kit appropriately.                          |
| **DNA is degraded.**                               | • Use fresh or frozen samples, whenever possible.                                
• Avoid the use of samples that have been stored extensively at room temperature (+15 to +25°C). |
| **Poor DNA yield**                                 | Shorten homogenization.                                                         |
| Sample did not contain enough material.            | Weigh tissue before use. Optimal results are obtained with 1 to 10 mg tissue. For some tissue types, more material (i.e., up to 25 mg) may be tried. |
| Too much sample material                           | Reduce amount of sample material to the values indicated in section “Sample Material”. |
| Inefficient homogenization                         | • Use recommended homogenization methods (see section “2.3 Pre-Isolation Steps for Fresh-Frozen Tissue”).  
• Cut tissue into small pieces prior to homogenization; apply more intensive homogenization. |
| Storage of samples was not optimal.                | • Use fresh or frozen samples, whenever possible.                                
• Avoid the use of samples that have been stored extensively at room temperature (+15 to +25°C). |
<p>| <strong>Poor DNA purity</strong>                                | Reduce amount of sample material to the values recommended in the sample material section “Sample Material”. |</p>
<table>
<thead>
<tr>
<th>Possible Cause</th>
<th>Recommendation</th>
</tr>
</thead>
</table>
| Poor PCR performance | **Too much DNA in PCR**  
- Check DNA concentration of eluates and adjust amount of eluate per PCR or dilute the eluate *(e.g., 1 in 10)*. Optimum: 1 to 100 ng/PCR, maximum: approx. 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. Optimum: 1 to 100 ng/PCR, maximum: approx. 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 *(e.g., Human Genomic DNA)*. |
|  | **PCR conditions are not optimal.**  
Optimize PCR, *e.g.*, include an initial denaturation of 30 s to 3 min 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 *(e.g., human genomic DNA)*.  
- If PCR product is found for control, design a smaller PCR amplicon for amplification of formalin fixed, paraffin-embedded tissue *(e.g., 100 to 400 bp; see Ref. 2)*. |
| Eluates show a slight red color. | **Minimal abrasion from magnetic particles**  
Centrifuge at low *g*-values (approx. 1,000 rpm) to remove fines.  
⚠️ The red color does not affect PCR using LightCycler® Instruments. |
5. Additional Information on this Product

5.1 How this Product Works

The MagNA Pure LC DNA Isolation Kit II (Tissue) is used with the MagNA Pure LC Instruments, to purify high-quality, undegraded genomic DNA from 1 to 32 samples of mammalian tissue. Isolated DNA meets the quality standards required for highly sensitive and quantitative PCR analysis using LightCycler® Instruments.

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, included 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. Purified DNA is eluted using a low salt buffer. 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 complete cell lysis and release of nucleic acids. Nucleases are denatured.
3. Proteinase K is added to the samples and proteins are digested.
DNA binds to the silica surface of the added MGPs, due to the chaotrope salt conditions, isopropanol and the high ionic strength of the Lysis/Binding Buffer.

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

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

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

Purified DNA is 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 DNA Isolation Kit II (Tissue) isolation procedure (“DNA II Tissue” protocol) are as follows:

**DNA Isolation performed using automation by the MagNA Pure LC Instruments**

1. Dispense Proteinase K to samples in the Sample Cartridge, mix and incubate.
2. Dispense all required reagents into the Processing Cartridge.
3. Add Lysis/Binding Buffer to the sample, then mix.
4. Transfer lysate into MGP suspension, mix and incubate.
5. Transfer MGPs into Wash Buffer I, mix, separate particles.
6. Transfer MGPs into Wash Buffer II, mix, separate particles.
7. Transfer MGPs into Wash Buffer III, mix, separate particles.
8. Transfer MGPs into the Elution Buffer, mix, incubate, 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® Carousel-Based Instrument. For typical experimental data, please refer to section “3. Results”.

5.3 References


6. Supplementary Information

6.1 Conventions

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

<table>
<thead>
<tr>
<th>Text Convention</th>
<th>Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbered stages labeled 1, 2 etc.</td>
<td>Stages in a process that usually occur in the order listed.</td>
</tr>
<tr>
<td>Numbered instructions labeled 1, 2 etc.</td>
<td>Steps in a procedure that must be performed in the order listed.</td>
</tr>
<tr>
<td>Asterisk *</td>
<td>Denotes a product available from Roche Applied Science.</td>
</tr>
</tbody>
</table>

**Symbols** In this Instruction Manual, the following symbols are used to highlight important information:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>❓</td>
<td>Information Note: Additional information about the current topic or procedure.</td>
</tr>
<tr>
<td>⚠️</td>
<td>Important Note: Information critical to the success of the procedure or use of the product.</td>
</tr>
</tbody>
</table>

**Abbreviations** In this Instruction Manual, the following abbreviations are used:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cp</td>
<td>crossing point</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variance</td>
</tr>
<tr>
<td>MGP</td>
<td>magnetic glass particle</td>
</tr>
</tbody>
</table>
6.2 Changes to Previous Version

- Editorial Changes
- Disclaimer of License updated

6.3 Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our Homepage, www.roche-applied-science.com and our Special Interest Sites including:


<table>
<thead>
<tr>
<th>Product</th>
<th>Pack Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Pure LC 2.0 Instrument</td>
<td>1 instrument plus accessories</td>
<td>05 197 686 001</td>
</tr>
<tr>
<td>MagNA Pure LC Cooling Block, LC Centrifuge Adapters</td>
<td>1 cooling block with 32 LightCycler® Centrifuge Adapters</td>
<td>12 190 664 001</td>
</tr>
<tr>
<td>MagNA Pure LC Cooling Block, LC Sample Carousel</td>
<td>1 cooling block</td>
<td>12 189 704 001</td>
</tr>
<tr>
<td>MagNA Pure LC Cooling Block, 96-well PCR Plate</td>
<td>1 cooling block</td>
<td>12 189 674 001</td>
</tr>
<tr>
<td>MagNA Pure LC 2.0 LightCycler® 480 Plate Adapter</td>
<td>1 adapter</td>
<td>05 323 983 001</td>
</tr>
<tr>
<td>Positioning Frame</td>
<td>only available with the MagNA Pure LC Disposables Starter Set</td>
<td>03 005 488 001</td>
</tr>
<tr>
<td>MagNA Pure LC Cartridge Seals</td>
<td>200 seals</td>
<td>03 118 827 001</td>
</tr>
<tr>
<td>MagNA Lyser Instrument</td>
<td>1 Instrument (220 V)</td>
<td>03 358 976 001</td>
</tr>
<tr>
<td></td>
<td>1 Instrument (110 V)</td>
<td>03 358 968 001</td>
</tr>
<tr>
<td></td>
<td>(plus Rotor and Rotor Cooling Block)</td>
<td></td>
</tr>
<tr>
<td>MagNA Lyser Green Beads</td>
<td>100 tubes</td>
<td>03 358 941 001</td>
</tr>
<tr>
<td>LightCycler® 480 Instrument II</td>
<td>1 instrument (96 well)</td>
<td>05 015 278 001</td>
</tr>
<tr>
<td></td>
<td>1 instrument (384 well)</td>
<td>05 015 243 001</td>
</tr>
<tr>
<td>LightCycler® 480 Multiwell Plate 96, white</td>
<td>5 × 10 plates (incl. sealing foil)</td>
<td>04 729 692 001</td>
</tr>
<tr>
<td>LightCycler® 480 Multiwell Plate 96, clear</td>
<td>5 × 10 plates (incl. sealing foil)</td>
<td>05 102 413 001</td>
</tr>
<tr>
<td>LightCycler® 2.0 Instrument</td>
<td>1 instrument plus accessories</td>
<td>03 531 414 001</td>
</tr>
<tr>
<td>LightCycler® 1.5 Instrument</td>
<td>1 instrument plus accessories</td>
<td>04 484 495 001</td>
</tr>
<tr>
<td>LightCycler® Capillaries (20 μl)</td>
<td>1 pack (5 boxes, each with 96 capillaries and stoppers)</td>
<td>04 929 292 001</td>
</tr>
<tr>
<td>LC Carousel Centrifuge 2.0</td>
<td>1 centrifuge plus rotor (230 V)</td>
<td>03 709 582 001</td>
</tr>
<tr>
<td></td>
<td>1 centrifuge plus rotor (115 V)</td>
<td>03 709 507 001</td>
</tr>
</tbody>
</table>
### Kits for DNA Isolation

<table>
<thead>
<tr>
<th>Product</th>
<th>Pack Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Pure LC DNA Isolation Kit I</td>
<td>1 kit (192 isolations)</td>
<td>03 003 990 001</td>
</tr>
<tr>
<td>MagNA Pure LC DNA Isolation Kit III (Bacteria, Fungi)</td>
<td>1 kit (192 isolations)</td>
<td>03 264 785 001</td>
</tr>
<tr>
<td>MagNA Pure LC DNA Isolation Kit – Large Volume</td>
<td>1 kit (96 to 288 isolations)</td>
<td>03 310 515 001</td>
</tr>
</tbody>
</table>

### Kits for RNA Isolation

<table>
<thead>
<tr>
<th>Product</th>
<th>Pack Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Pure LC RNA Isolation Kit – High Performance</td>
<td>1 kit (192 isolations)</td>
<td>03 542 394 001</td>
</tr>
<tr>
<td>MagNA Pure LC RNA Isolation Kit III (Tissue)</td>
<td>1 kit (192 isolations)</td>
<td>03 330 591 001</td>
</tr>
<tr>
<td>MagNA Pure LC mRNA HS Kit</td>
<td>1 kit (192 isolations)</td>
<td>03 267 393 001</td>
</tr>
</tbody>
</table>

### Kits for Total Nucleic Acid Isolation

<table>
<thead>
<tr>
<th>Product</th>
<th>Pack Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>MagNA Pure LC Total Nucleic Acid Isolation Kit</td>
<td>1 kit (192 isolations)</td>
<td>03 038 505 001</td>
</tr>
<tr>
<td>MagNA Pure LC Total Nucleic Acid Isolation Kit - Large Volume</td>
<td>1 kit (192 isolations)</td>
<td>03 264 793 001</td>
</tr>
<tr>
<td>MagNA Pure LC Total Nucleic Acid Isolation Kit - High Performance</td>
<td>1 kit (96 to 288 isolations)</td>
<td>05 323 738 001</td>
</tr>
</tbody>
</table>

### Associated Reagents

<table>
<thead>
<tr>
<th>Product</th>
<th>Pack Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNase A</td>
<td>25 mg</td>
<td>10 109 142 001</td>
</tr>
<tr>
<td></td>
<td>100 mg</td>
<td>10 109 169 001</td>
</tr>
<tr>
<td>Human Genomic DNA</td>
<td>100 μg</td>
<td>11 691 112 001</td>
</tr>
</tbody>
</table>

### 6.4 Trademarks

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### 6.5 Disclaimer of License

NOTICE TO PURCHASER:
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### 6.6 Regulatory Disclaimer

For general laboratory use.
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We also want you to contact us if you have suggestions for enhancing RAS product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to RAS and the worldwide research community.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our Online Technical Support Site at:

www.roche-applied-science.com/support

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- in-depth Technical Manuals
- Lab FAQS: Protocols and references for life science research
- our quarterly Biochemica Newsletter
- Material Safety Data Sheets
- Pack Inserts and Product Instructions

or to request hard copies of printed materials.