Agarose Gel DNA Extraction Kit

For the elution of DNA fragments from agarose gels

Cat. No. 11 696 505 001 1 kit
up to 100 reactions

Store the kit at +15 to +25°C.
1. General Information

1.1. Contents

<table>
<thead>
<tr>
<th>Vial/Bottle</th>
<th>Cap</th>
<th>Label</th>
<th>Function / Description</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>colorless</td>
<td>Silica Matrix</td>
<td>Pretreated spherical silica matrix sufficient for 100 standard reactions.</td>
<td>White suspension</td>
</tr>
<tr>
<td>2</td>
<td>colorless</td>
<td>Agarose Solubilization Buffer</td>
<td>Agarose Solubilization Buffer suitable for solubilization of TAE or TBE buffer agarose gels. Contains sodium perchlorate.</td>
<td>60 ml</td>
</tr>
<tr>
<td>3</td>
<td>green</td>
<td>Nucleic Acid Binding Buffer</td>
<td>Binding Buffer <strong>Contains sodium perchlorate.</strong></td>
<td>100 ml</td>
</tr>
<tr>
<td>4</td>
<td>blue</td>
<td>Washing Buffer</td>
<td>Washing Buffer <strong>Add 80 ml absolute ethanol before use!</strong></td>
<td>20 ml</td>
</tr>
</tbody>
</table>

1.2. Storage and Stability

Storage Conditions (Product)

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

1.3. Additional Equipment and Reagents Required

Optional:
- Agarose MP*
- Agarose LE*
- Agarose MS*

1.4. Application

The Agarose Gel DNA Extraction Kit is designed for the efficient isolation of DNA fragments from TAE or TBE agarose gels. The kit is applicable for DNA isolation from standard agarose gels (e.g., Agarose MP* and Agarose LE*). DNA fragments isolated with the Agarose Gel DNA Extraction Kit are efficiently ligated into plasmid cloning vectors or labeled to high specific activity using either random primed labeling or nick translation.
2. How to Use this Product

2.1. Protocols

Isolation of Electrophoretically Separated DNA Fragments with the Agarose Gel DNA Extraction Kit

⚠️ Make sure that 80 ml absolute ethanol has been added to the Washing Buffer prior to the first use (Vial 4, blue cap).

1. Separate the DNA of interest in an agarose gel of suitable concentration.
   - Use either 1x TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) or 1x TBE running buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0).

2. After sufficient separation, cut out the interesting DNA fragment with a sharp scalpel or razor blade.
   - Take care to leave as much of the agarose gel as possible.
   - Transfer in a preweighted reaction tube.

3. Use 300 μl of the Agarose Solubilization Buffer (Vial 2) per 100 mg of agarose gel.
   - Apply 600 μl if the agarose concentration used for the gel preparation is more than 2%.

4. Resuspend the Silica Suspension (Vial 1) until a homogeneous suspension is obtained.
   - Add 10 μl of the silica suspension to the sample.
   - If the sample contains more than 2.5 μg DNA, increase the amount of silica suspension by 4 μl for each additional μg of DNA.

5. Incubate the mixture for 10 min at +56 to +60°C and vortex every 2 – 3 min.

6. Centrifuge in a tabletop centrifuge for 30 s at maximal speed and discard the supernatant.

7. Resuspend the matrix containing the DNA with 500 μl Nucleic Acid Binding Buffer (Vial 3, green cap) on a vortex mixer.
   - Centrifuge and discard supernatant as before.

8. Wash the pellet with 500 μl Washing Buffer (Vial 4, blue cap).
   - Centrifuge and discard supernatant as before.
   - Repeat this step once.

9. Remove all the liquid with a pipette, then invert the tube on an adsorbent tissue and let dry at room temperature for 15 min.
   - Do not apply vacuum since overdrying may lead to lower yields. The matrix color turns to bright white when dry.

10. Use 20 – 50 μl of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0 – 8.5) or double-distilled water (pH 8 – 8.5) for the elution of DNA.
   - The elution efficiency is increased with higher volume of elution buffer applied or application of two elution cycles with 2 × 25 μl.
     - Vortex and incubate for 10 min at +15 to +25°C or +56 to +60°C.
     - Vortex every 2 – 3 min.
     - After centrifugation at maximum speed for 30 s, transfer the DNA-containing solution to a new reaction tube taking care not to carry over any matrix material.
2.2. Other Parameters

Inhibition

No inhibition of digestion with restriction endonucleases is observed according to the current Quality Control procedures.
3. Results

The appropriate size range for isolation of DNA fragments with the Agarose Gel DNA Extraction Kit is from 400 bp to 5 kb with a reproducible recovery of about 80%.

Typical recoveries with smaller and larger fragment sizes:
- 20 bp – 55%
- 40 bp – 68%
- 120 bp – 76%
- 200 bp – 80%
- 8 – 9 kbp – 75%
- >10 kbp – < 60%

If lower recovery rates are acceptable, larger DNA fragments (up to 100 kb) can be isolated without degradation or shearing of the DNA due to the uniformity of the spherical particles. For fragments >10 kb, prewarm the elution buffer, perform the elution in a water bath, and increase the elution time up to 15 to 20 minutes with intermediate vortexing.
## 4. Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Cause</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low yield</td>
<td>Incomplete agarose gel solubilization.</td>
<td>Check temperature.</td>
</tr>
<tr>
<td></td>
<td>Elution buffer has acidic pH.</td>
<td>Check pH to be 8.0 – 8.5.</td>
</tr>
<tr>
<td></td>
<td>Larger fragments (&gt;5 kb) are adsorbed more strongly to the matrix.</td>
<td>Elute at +56 to +60°C for 15 – 20 min.</td>
</tr>
<tr>
<td></td>
<td>Pellet is too dry.</td>
<td>Elute at +56 to +60°C.</td>
</tr>
<tr>
<td>Inhibition of subsequent enzymatic reactions</td>
<td>Residual ethanol from Washing Buffer.</td>
<td>Complete removal of Washing Buffer. Allow sufficient air drying.</td>
</tr>
<tr>
<td></td>
<td>Incomplete removal of the Silica Matrix.</td>
<td>Recentrifuge DNA solution and transfer to a new reaction tube.</td>
</tr>
</tbody>
</table>
5. Additional Information on this Product

5.1. Test Principle

Nucleic acids bind specifically to the surface of glass or silica materials in the presence of a chaotropic salt (Vogelstein, B. & Gillespie, D., 1979). The binding reaction occurs due to the disruption of the organized structure of water molecules and the interaction with the nucleic acids. Thus, the adsorption to the specifically pretreated spherical silica matrix is favored. Since the binding process is specific for nucleic acids, the bound material can be separated and purified from impurities (e.g., salts and proteins, by a simple washing step). Nucleic acids elute from the matrix in a low-salt buffer or water.

The uniformity of the spherical particles enables the isolation of intact large DNA fragments, up to 100 kbp in length. Furthermore, the narrow size distribution and the absence of fine material that might inhibit subsequent enzymatic reactions, add favorable to the high binding capacity of the silica matrix also for small DNA fragments. Thus, even oligonucleotides larger than 20 nucleotides can be isolated from agarose gels.

5.2. Quality Control

DNA molecular weight marker with fragments from 500 bp – 3 kb and the eluate from the kit protocol are separated in an agarose gel. The recovery of the isolated DNA fragments were at least 85% referring to 500 bp and 75% referring to 3 kb.
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:

<table>
<thead>
<tr>
<th>Text convention and symbols</th>
<th>Information Note: Additional information about the current topic or procedure.</th>
</tr>
</thead>
<tbody>
<tr>
<td>! Important Note: Information critical to the success of the current procedure or use of the product.</td>
<td></td>
</tr>
<tr>
<td>① ② ③ etc.</td>
<td>Stages in a process that usually occur in the order listed.</td>
</tr>
<tr>
<td>① ② ③ etc.</td>
<td>Steps in a procedure that must be performed in the order listed.</td>
</tr>
<tr>
<td>* (Asterisk)</td>
<td>The Asterisk denotes a product available from Roche Diagnostics.</td>
</tr>
</tbody>
</table>

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Ordering Information

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

<table>
<thead>
<tr>
<th>Product</th>
<th>Pack Size</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagents, kits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agarose LE</td>
<td>100 g</td>
<td>11 685 660 001</td>
</tr>
<tr>
<td></td>
<td>500 g</td>
<td>11 685 678 001</td>
</tr>
<tr>
<td>Agarose MS</td>
<td>100 g</td>
<td>11 816 586 001</td>
</tr>
<tr>
<td></td>
<td>500 g</td>
<td>11 816 594 001</td>
</tr>
<tr>
<td>Agarose MP</td>
<td>500 g bulk</td>
<td>03 573 788 001</td>
</tr>
<tr>
<td></td>
<td>100 g</td>
<td>11 388 983 001</td>
</tr>
<tr>
<td></td>
<td>500 g</td>
<td>11 388 991 001</td>
</tr>
</tbody>
</table>
6.4. Trademarks
All third party 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 life science research only. Not for use in diagnostic procedures.

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.