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Not for use in diagnostic procedures.



# Agarose Gel DNA Extraction Kit

 **Version: 13**

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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.**

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# 1. General Information

## 1.1. Contents

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

## 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.

**i** 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.

**i** 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.

**i** 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

Observation	Possible Cause	Recommendation
Low yield	Incomplete agarose gel solubilization.	Check temperature.
	Residual ethanol from Washing Buffer.	Complete removal of Washing Buffer. Allow sufficient air drying.
	Elution buffer has acidic pH.	Check pH to be 8.0 – 8.5.
	Larger fragments (>5 kb) are adsorbed more strongly to the matrix.	Elute at +56 to +60°C for 15 – 20 min.
	Pellet is too dry.	Elute at +56 to +60°C.
Inhibition of subsequent enzymatic reactions	Residual ethanol from Washing Buffer.	Complete removal of Washing Buffer. Allow sufficient air drying.
	Incomplete removal of the Silica Matrix.	Recentrifuge DNA solution and transfer to a new reaction tube.
	Incomplete removal of Nucleic Acid Binding Buffer.	Chaotropic salts inhibit enzyme reaction. Remove Binding Buffer completely.

## 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:

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

### 6.2. Changes to previous version

Editorial changes.

### 6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents , kits		
Agarose LE	100 g	11 685 660 001
	500 g	11 685 678 001
Agarose MS	100 g	11 816 586 001
	500 g	11 816 594 001
Agarose MP	500 g bulk	03 573 788 001
	100 g	11 388 983 001
	500 g	11 388 991 001

## 6. Supplementary Information

### 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: **List of LifeScience products**

### 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

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

To call, write, fax, or email us, visit **sigma-aldrich.com** and select your home country. Country-specific contact information will be displayed.

