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QIAEX® II Handbook

For DNA extraction from agarose and polyacrylamide gels and for desalting and concentrating DNA from solutions

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Contents

Kit Contents

QIAEX II Gel Extraction Kit	(150)	(500)
Catalog no.	20021	20051
No. of preparations* (10 µl QIAEX II per prep)	150	500
QIAEX II Suspension	3 x 0.5 ml	10 x 0.5 ml
Buffer QX1† (with pH indicator)	2 x 100 ml	6 x 100 ml
Buffer PE (concentrate)	4 x 10 ml	3 x 55 ml
Quick-Start Protocol	1	1

* Routine purifications from gel slices >100 mg, which contain fragments <100 bp or where the gels are >2% agarose and require additional Buffer QX1 to perform the full number of extractions (see ordering information, page 21).

† Buffer QX1 contains chaotropic salt, which is an irritant. Take appropriate laboratory safety precautions and wear gloves when handling.

Storage

The QIAEXII Gel Extraction Kit should be stored dry at room temperature (15–25°C). When stored under these conditions and handled appropriately, the kit can be stored for at least 12 months without showing any reduction in performance, capacity, or quality of separation.

Intended Use

The QIAEX II Gel Extraction Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAEX II Gel Extraction Kit is tested against predetermined specifications to ensure consistent product quality.

Product Specifications

Maximum binding capacity: 5 µg DNA per 10 µl QIAEX II suspension

Recovery: 60–95% of DNA fragments 40 bp – 50 kb

Minimum elution volume: 20 µl

Introduction

The QIAEX II Gel Extraction Kit is designed to extract and purify DNA from any agarose gel in either TAE (Tris-acetate/EDTA) or TBE (Tris-borate/EDTA) buffer, without phenol extraction or ethanol precipitation. QIAEX II silica particles have been optimized to enhance recovery of very small and very large DNA fragments. DNA molecules of 40 bp to 50 kb are adsorbed to QIAEX II particles in the presence of high salt. Non-nucleic acid impurities such as agarose, proteins, salts, and ethidium bromide are removed during washing steps. The pure DNA is efficiently eluted in just 20 µl of Tris buffer or water, and is suitable for most subsequent applications; for example, restriction digestion, labeling, ligation, PCR, sequencing, and microinjection (see "Applications using QIAEX II purified fragments", page 10).

Principle

With the QIAEX II Gel Extraction Kit, extraction and purification of DNA fragments is based on solubilization of agarose and selective, quantitative adsorption of nucleic acids to the QIAEX II silica particles in the presence of high salt. Elution of DNA is accomplished with a low-salt solution such as Tris buffer or water.

Solubilization of agarose without sodium iodide (NaI)

The optimized Buffer QX1 in the QIAEX II Gel Extraction Kit efficiently solubilizes agarose, and does not contain NaI. Residual NaI may be difficult to remove from DNA samples, and reduces the efficiency of subsequent enzymatic reactions such as blunt-end ligation.

The standard QIAEX II Gel Extraction protocol is used to extract DNA from 0.3–2% standard or low-melt agarose gels in TAE or TBE buffer. The concentration of agarose in the gel determines the size range of DNA molecules that can be resolved (Table 1).

Table 1. Separation range in TAE gels containing different concentrations of agarose (1)

Agarose	Size of DNA fragments separated
0.3%	5 – 60 kb
0.5%	1 – 30 kb
0.7%	0.8 – 12 kb
1.0%	0.5 – 10 kb
1.2%	0.4 – 7 kb
1.5%	0.2 – 3 kb
2.0%	0.05 – 2 kb

A typical agarose gel slice is solubilized by adding 3 volumes of Buffer QX1 to 1 volume of gel (e.g., 300 µl of Buffer QX1 is added to 100 mg gel slice) and incubating at 50°C for 10 minutes. The high concentration of a chaotropic salt in Buffer QX1 disrupts hydrogen bonding between sugars in the agarose polymer, allowing solubilization of the gel slice. In addition, the high salt concentration dissociates DNA-binding proteins from the DNA fragments.

A ≤ 2% agarose gel slice is normally solubilized within 2–3 minutes with Buffer QX1 at 50°C. The incubation with QIAEX II is extended to 10 minutes to complete the adsorption of DNA to the QIAEX II particles.

Efficient DNA adsorption to QIAEX II — salt and pH dependence

Adsorption of DNA to glass, silica, or diatomaceous earth in high salt is a well known phenomenon (2). Incubation of a DNA solution in a highly electrolytic environment with large anions (e.g., from chaotropic salts) causes a modification in the structure of water (3), forcing the DNA to adsorb to the silica particles. Adsorption of fragments smaller than 100 bp is enhanced by increasing the salt concentration, while fragments larger than 4 kb are adsorbed at lower salt concentrations.

Adsorption of DNA to silica also depends on pH. Adsorption efficiency is typically 95% if the pH is ≤7.5, and is drastically reduced at higher pH (Figure 1). If the pH of the binding mixture is >7.5, the optimal pH for DNA binding can be achieved by adding a small volume of 3 M sodium acetate, pH 5.0.

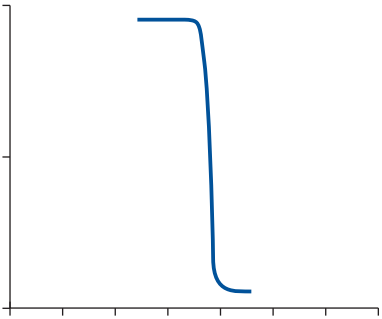

Figure 1. pH dependence of DNA adsorption to silica. One microgram of a 2.9 kb fragment was adsorbed at different pH values and eluted with 10 mM Tris·Cl, pH 8.5. The graph shows percentage DNA recovery, reflecting the relative adsorption efficiency, versus pH of adsorption.

QIAEX II Gel Extraction Procedure

pH indicator in solubilization and binding Buffer QX1

Buffer QX1 in the QIAEX II Gel Extraction Kit is used for solubilization of agarose gel slices and binding of DNA to QIAEX II silica particles. Buffer QX1 contains a pH indicator, allowing easy determination of the optimal pH for DNA binding. DNA adsorption requires a pH ≤7.5, and the pH indicator appears yellow in this range. If the pH is >7.5, which can occur if the agarose gel electrophoresis buffer is frequently used or incorrectly prepared, the binding mixture turns orange or violet (Figure 2). This means that the pH of the sample exceeds the buffering capacity of Buffer QX1 and DNA adsorption will be inefficient. In this case, the pH of the binding mixture can easily be corrected by addition of a small volume of 3 M sodium acetate, pH 5.0, before proceeding with the protocol.

The color of the binding mixture allows easy visualization of any unsolubilized agarose, ensuring complete solubilization and maximum yields. The indicator dye does not interfere with DNA binding and is completely removed during the cleanup procedure.

Figure 2. Indicator dye in solubilization and binding Buffer QX1 identifies optimal pH for DNA binding.

Washing

DNA molecules bind to the QIAEX II particles during the adsorption step, and non-nucleic acid impurities such as agarose, proteins, ethidium bromide, and salts remain in the supernatant. A high salt wash with Buffer QX1 removes residual agarose, and two washes with ethanol-containing Buffer PE efficiently remove salt contaminants. All traces of supernatant must be carefully removed at each step to eliminate impurities and reduce buffer carryover.

Washed QIAEX II particles carrying adsorbed DNA are pelleted and air-dried at room temperature (15–25°C) for 10–15 minutes. Drying the pellet is necessary to remove all traces of residual ethanol, which may interfere with subsequent enzymatic reactions.

Elution in low-salt solutions

Elution efficiency depends on pH and salt concentration. Contrary to adsorption, elution is best under basic conditions and low salt concentrations when using QIAEX II. DNA is typically eluted with 20 µl of 10 mM Tris·Cl, pH 8.5, or with water. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water to elute, make sure that the pH value is within this range. In addition, DNA must be stored at –20°C when eluted with water, since DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

Elution efficiency also depends on temperature. DNA fragments smaller than 4 kb are recovered with 70–95% efficiency when eluted at room temperature (15–25°C) with a 5-min incubation. For efficient elution of DNA fragments larger than 4 kb, the incubation temperature should be increased to 50°C.

After elution, QIAEX II is pelleted by centrifugation. The supernatant containing pure DNA is easily removed from the QIAEX II pellet, ready for use in subsequent applications.

Applications using QIAEX II purified fragments

DNA purified with QIAEX II is suitable for various applications, for example, restriction digestion, labeling, ligation, transformation, PCR, sequencing, in vitro transcription, and microinjection. Some applications, such as sequencing, blunt-end ligation, in vitro transcription, or microinjection, are highly sensitive to resin carryover and residual salts, and therefore all traces of supernatant must be carefully removed at each wash step. After elution, the supernatant containing the pure DNA should be carefully removed from the QIAEX II pellet. If necessary, the supernatant may be centrifuged a second time to ensure complete removal of all QIAEX II particles.

For sensitive downstream applications such as fluorescent sequencing, The QIAquick® Gel Extraction Kit (see ordering information, page 21) is recommended. The QIAquick Gel Extraction Kit combines a convenient microspin format with a specially designed silica membrane. The quality of the purified DNA is less dependent on the handling procedures.

Protocol: Extraction of 40 bp to 50 kb DNA fragments from Agarose Gels

This protocol is designed for the extraction of 40 bp to 50 kb DNA fragments from 0.3–2% standard or low-melt agarose gels in TAE or TBE buffers.

Important points before starting

- The yellow color of Buffer QX1 indicates a pH ≤7.5.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- A heating block or water bath at 50°C is required.
- 3 M sodium acetate, pH 5.0, may be necessary.
- All centrifugation steps are at maximum speed (≥10,000 x *g*, ~13,000 rpm) in a conventional, table-top microcentrifuge.
- For DNA fragments larger than 10 kb, mix by gently flicking the tube to avoid shearing the DNA. Do not vortex the tube.

Procedure

1. **Excise the DNA band from the agarose gel with a clean, sharp scalpel.**

   Minimize the size of the gel slice by removing excess agarose. Use a 1.5 ml microfuge tube for processing up to 250 mg agarose.

2. **Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QX1 to 1 volume of gel for DNA fragments 100 bp – 4 kb; otherwise, follow the table below.**

   For example, add 300 µl of Buffer QX1 to each 100 mg of gel.

Table 2. Addition of Buffer QXI

DNA fragments <100 bp	Add 6 volumes of Buffer QX1
DNA fragments >4 kb	Add 3 volumes of Buffer QX1 plus 2 volumes of H_2O
>2% or Metaphor agarose gels	Add 6 volumes of Buffer QX1

3. **Resuspend QIAEX II by vortexing for 30 s. Add QIAEX II to the sample according to the table below and mix.**

Table 3. Addition of QIAEX II

≤ 2 µg DNA	Add 10 µl of QIAEX II
2–10 µg DNA	Add 30 µl of QIAEX II
Each additional 10 µg DNA	Add additional 30 µl of QIAEX II

4. **Incubate at 50°C for 10 min to solubilize the agarose and bind the DNA. Mix by vortexing* every 2 min to keep QIAEX II in suspension. Check that the color of the mixture is yellow.**

   If the color of the mixture is orange or purple, add 10 µl 3 M sodium acetate, pH 5.0, and mix. The color should turn to yellow. The incubation should then be continued for an additional 5 min at least.

   The adsorption of DNA to QIAEX II particles is only efficient at pH ≤ 7.5. Buffer QX1 now contains a pH indicator which is yellow at pH ≤ 7.5, and orange or violet at higher pH, allowing easy determination of the optimal pH for DNA binding.

5. **Centrifuge the sample for 30 s and carefully remove supernatant with a pipet.**

6. **Wash the pellet with 500 µl of Buffer QX1.**

   Resuspend the pellet by vortexing.* Centrifuge the sample for 30 s and remove all traces of supernatant with a pipet. This wash step removes residual agarose contaminants.

7. **Wash the pellet twice with 500 µl of Buffer PE.**

   Resuspend the pellet by vortexing*. Centrifuge the sample for 30 s and carefully remove all traces of supernatant with a pipet. These washing steps remove residual salt contaminants.

8. **Air-dry the pellet for 10–15 min or until the pellet becomes white.**

   If 30 µl of QIAEX II suspension is used, air-dry the pellet for approximately 30 min. Do not vacuum dry, as this may cause overdrying. Overdrying the QIAEX II pellet may result in decreased elution efficiency.

9. **To elute DNA, add 20 µl of 10 mM Tris·Cl, pH 8.5 or H_2O and resuspend the pellet by vortexing*. Incubate according to the table in the next page.**

* For fragments larger than 10 kb, resuspend the pellet by inverting and flicking the tube. Vortexing can cause shearing of large DNA fragments.

Table 4. Incubation conditions

DNA fragments ≤4 kb	Incubate at room temp. for 5 min
DNA fragments 4–10 kb	Incubate at 50°C for 5 min
DNA fragments >10 kb	Incubate at 50°C for 10 min

Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

10. **Centrifuge for 30 s. Carefully pipet the supernatant into a clean tube.**

    The supernatant now contains the purified DNA.

11. **Optional: repeat steps 9 and 10 and combine the eluates.**

    A second elution step will increase the yield by approximately 10–15%.

Protocol: Extraction of DNA fragments from Polyacrylamide Gels

Important points before starting:

- The yellow color of Buffer QX1 indicates a pH ≤7.5.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- A heating block or water bath at 50°C is required.
- 3 M sodium acetate, pH 5.0, may be necessary.
- All centrifugation steps are at maximum speed (≥10,000 x *g*, ~13,000 rpm) in a conventional, table-top microcentrifuge.
- Prepare diffusion buffer: 0.5 M ammonium acetate; 10 mM magnesium acetate; 1 mM EDTA, pH 8.0; 0.1% SDS.
- A disposable plastic column or a syringe barrel containing either a Whatman® GF/C filter or packed, siliconized glass wool will be required for each extraction.

Procedure

1. **Excise the gel slice containing the DNA band with a clean, sharp scalpel.**

   Minimize the size of the gel slice by removing excess polyacrylamide.
2. **Weigh the gel slice. Add 1–2 volumes of diffusion buffer to 1 volume of gel (i.e., 100–200 µl for each 100 mg of gel).**
3. **Incubate at 50°C for 30 min.**
4. **Microcentrifuge the sample for 1 min.**
5. **Carefully remove the supernatant using a pipet or a drawn-out Pasteur pipet. Pass the supernatant through a disposable plastic column or a syringe containing either a Whatman GF/C filter or packed, siliconized glass wool to remove any residual polyacrylamide.**
6. **Calculate the approximate volume of the recovered supernatant.**
7. **For DNA fragments <100 bp, add 6 volumes of Buffer QX1 to 1 volume of sample. Otherwise, add 3 volumes Buffer QX1. Check that the color of the mixture is yellow.**

   If the color of the mixture is orange or purple, add 10 µl 3 M sodium acetate, pH 5.0, and mix. The color should turn to yellow.

   The adsorption of DNA to QIAEX II particles is only efficient at pH ≤7.5. Buffer QX1 now contains a pH indicator which is yellow at pH ≤7.5, and orange or violet at higher pH, allowing easy determination of optimal pH for DNA binding.
8. **Resuspend QIAEX II by vortexing for 30 s.**

9. **Add 10 µl of QIAEX II and mix. Incubate at room temparature (15–25°C) for 10 min. Vortex every 2 min to keep QIAEX II in suspension.**
10. **Centrifuge the sample for 30 s and remove supernatant.**
11. **Wash the pellet twice with 500 µl of Buffer PE.**
12. **Air-dry the pellet for 10–15 min or until the pellet becomes white.**

    Do not vacuum dry, as this may cause overdrying. Overdrying the QIAEX II pellet may result in decreased elution efficiency.
13. **To elute DNA, add 20 µl of 10 mM Tris·Cl, pH 8.5, or H_2O and resuspend the pellet by vortexing. Incubate for 5 min at room temperature.**

    Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.
14. **Centrifuge for 30 s. Carefully transfer the supernatant into a clean tube.**

    The supernatant now contains purified DNA.
15. **Optional: repeat steps 13 and 14 and combine the eluates.**

    A second elution step will increase the yield by approximately 10–15%.

Protocol: Desalting and Concentrating DNA Solutions

QIAEX II Gel Extraction Kit can be used to purify and concentrate DNA fragments from 40 bp to 50 kb from aqueous solutions, without phenol extraction or ethanol precipitation. Enzymes such as phosphatases, restriction endonucleases, polymerases, as well as dNTPs, and salts are removed. For direct purification from PCR reactions, use the QIAquick PCR Purification Kit (see ordering information, page 21) to recover PCR products and efficiently remove primers.

Important points before starting

- The yellow color of Buffer QXI indicates a pH ≤7.5.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are at maximum speed (≥10,000 x *g*, ~13,000 rpm) in a conventional table-top microcentrifuge.
- For DNA fragments larger than 10 kb, mix by gently flicking the tube to avoid shearing the DNA. Do not vortex the tube.

Procedure

1. **Transfer the sample to a colorless tube. Add 3 volumes of Buffer QX1 to 1 volume of sample.**

   For DNA fragments 100 bp–4 kb; otherwise follow the table below.

Table 5. Addition of Buffer QX1

DNA fragments <100 bp	Add 6 volumes of Buffer QX1
DNA fragments >4 kb	Add 3 volumes of Buffer QX1 plus 2 volumes of H_2O

2. **Check that the color of the sample mixture is yellow.**

   If the color of the mixture is orange or purple, add 10 µl 3 M sodium acetate, pH 5.0, and mix. The color should now be yellow.

   The adsorption of DNA to QIAEX II particles is only efficient at pH ≤7.5. Buffer QX1 now contains a pH indicator, which is yellow at pH ≤7.5, and orange or violet at higher pH, allowing easy determination of the optimal pH for DNA binding.

3. **Resuspend QIAEX II by vortexing 30 s.**

4. **Add 10 µl of QIAEX II per 5 µg of DNA and mix*. Incubate at room temperature (15–25°C) for 10 min. Mix every 2 min to keep QIAEX II in suspension.**
5. **Centrifuge the sample for 30 s and remove supernatant.**
6. **Wash the pellet twice with 500 µl of Buffer PE.**
7. **Air-dry the pellet for 10–15 min or until the pellet becomes white.**

   Do not vacuum dry, as this may cause overdrying. Overdrying the QIAEX II pellet may result in decreased elution efficiency.
8. **To elute DNA, add 20 µl of 10 mM Tris·Cl, pH 8.5, or H_2O and resuspend the pellet by vortexing.* Incubate according to the table below.**

Table 6. Incubation conditons

DNA fragments ≤ 4 kb	Incubate at room temp. for 5 min
DNA fragments 4–10 kb	Incubate at 50°C for 5 min
DNA fragments >10 kb	Incubate at 50°C for 10 min

Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water for elution, make sure that the pH is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

9. **Centrifuge for 30 s. Carefully transfer the supernatant into a clean tube.**

   The supernatant now contains purified DNA.
10. **Optional: repeat steps 8 and 9 and combine the eluates.**

    A second elution step will increase the yield by approximately 10–15%.

* For fragments larger than 10 kb, resuspend the pellet by inverting and flicking the tube. Vortexing can cause shearing of large DNA fragments.

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

		Comments and suggestions
Poor DNA recovery		
a)	Ethanol was not added to concentrated Buffer PE before use	Repeat procedure with correctly prepared Buffer PE.
b)	pH of electrophoresis buffer too high (binding mixture turns orange or violett).	The electrophoresis buffer has been repeatedly used or incorrectly prepared, resulting in a sample pH that exceeds the buffering capacity of Buffer QX1 and leads to inefficient DNA binding. Add 10 µl of 3 M sodium acetate, pH 5.0, to the sample and mix. The color of the mixture should turn yellow indicating the correct pH for DNA binding. For binding mixtures with only small color changes (slight orange color), add the sodium acetate.
c)	Inappropriate elution buffer	DNA will only be eluted in the presence of low salt buffer (e.g., 10 mM Tris·Cl, pH 8.5) or water. Check the pH and salt concentration of the elution buffer.
d)	Incomplete solubilization of gel slice in Buffer QX1	Mix the tube every 2 min during the solubilization step. Large gel slices may require a few extra minutes at 50°C to solubilize. DNA will remain in any undissolved agarose.
e)	Low salt concentration during adsorption	Sufficient salt must be present during the adsorption step. Weigh the gel slice weight accurately and add the appropriate amount of Buffer QX1.

		Comments and suggestions
f)	Insufficient amount of QIAEX II	Enough QIAEX II must be present at the adsorption step to bind DNA. See Table 3 on page 12 for details.
g)	Buffer QX1 incompletely removed before wash step with Buffer PE	If Buffer QX1 is not completely removed, the subsequent Buffer PE washing steps may not efficiently remove all traces of salt. The presence of high concentrations of salt may prevent complete elution of DNA from the QIAEX II particles. Additionally, residual salt may result in poor performance of the DNA in subsequent enzymatic reactions.
h)	Over dried QIAEX II pellet	Do not use a vacuum dryer to dry the QIAEX II pellet. If pellet is dried too extensively, the DNA may irreversibly bind to the QIAEX II particles, resulting in decreased recovery.
i)	Insufficient volume of elution buffer	Increase volume of elution buffer in proportion to amount of extra QIAEX II added. An optional extra elution step will increase yield by approximately 10–15%.
j)	Incorrect volume of Buffer QX1 added for solubilization	See Table 2 on page 11 for details.
DNA does not perform well in subsequent reactions		
a)	Incomplete removal of Buffer QX1 supernatant	Salt from Buffer QX1 may be carried over and inhibit subsequent enzymatic reactions. Wash twice with Buffer PE.
b)	Incomplete removal of Buffer PE supernatant	Residual ethanol may reduce elution efficiency. Carefully remove all Buffer PE to enable pellet to dry sufficiently before elution.
c)	Incomplete removal of QIAEX II particles after elution	QIAEX II particles may bind proteins under low salt conditions, and reduce enzyme activity in subsequent reactions. Centrifuge again and take the supernatant to remove all traces of QIAEX II.
DNA contains residual ethanol (samples float out of wells of agarose gels)		
a)	QIAEX II pellet was under dried	Buffer PE was not completely removed by drying. Dry the DNA eluate briefly in a vacuum dryer to remove residual ethanol.

		Comments and suggestions
DNA appears to "smear" on an agarose gel.		
a)	Sample well is overloaded	Smiling and smearing of DNA bands may occur when too much sample is loaded in one well. Use less DNA per well or use a larger well.
b)	Vigorous handling of DNA	Vortexing and vigorous pipetting of fragments above 10 kb should be avoided throughout. QIAEX II can be easily resuspended by flicking the microfuge tube. Very large DNA fragments can be protected from shearing by keeping the QIAEX II pellet intact during the washing steps. The wash buffers should remain on the QIAEX II pellet for 5 min to allow complete diffusion.
c)	Eluate contains denatured ssDNA	Denatured ssDNA appears as a smaller smeared band on an analytical gel. Use the eluted DNA to prepare the subsequent enzymatic reaction but omit the enzyme. To reanneal the ssDNA, incubate the reaction mixture at 95°C for 2 min, and allow the tube to cool slowly to room temparature (15–25°C). Add the enzyme and proceed as usual. Alternatively, the DNA can be eluted in 10 mM Tris buffer containing 10 mM NaCl. The salt and buffering agent promote the renaturation of DNA strands. However, the salt concentration of the eluate must then be considered for subsequent applications.

References

1. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.
2. Vogelstein, B., and Gillespie, D. (1979) Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA ***76***, 615–619.
3. Hamaguchi, K., and Geiduschek, E.P. (1962) The effect of electrolytes on the stability of deoxyribonucleate helix. J. Am. Chem. Soc. ***84***, 1329–1337.

Ordering Information

Product	Contents	Cat. no.
QIAEX II Gel Extraction Kit (150)	For 150 extractions:* 3 x 0.5 ml QIAEX II Suspension, Buffers	20021
QIAEX II Gel Extraction Kit (500)	For 500 extractions:* 5 x 1.0 ml QIAEX II Suspension, Buffers	20051
QIAEX II Suspension	3 x 0.5 ml QIAEX II Suspension	20902
Buffer QX1	500 ml Solubilization and Binding Buffer (with pH indicator)	20912
Buffer PE	100 ml Wash Buffer (concentrate; final volume 500 ml)	19065
Related products		
QIAquick PCR Purification Kit (50)†	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28104
QIAquick Gel Extraction Kit (50)†	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28704
QIAquick Nucleotide Removal Kit (50)†	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28304
MinElute® PCR Purification Kit (50)†	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28004
MinElute Gel Extraction Kit (50)†	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28604
MinElute Reaction Cleanup Kit (50)†	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28204

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* Routine purifications from gel slices >100 mg, which contain fragments <100 bp or where the gels contain >2% agarose and require additional Buffer QX1 to perform the full number of extractions.

† Larger kit sizes/formats available; see www.qiagen.com.

Notes

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