QIAGEN Supplementary Protocol:

High-throughput gel extraction using the QIAquick® 96 PCR Purification Kit

This protocol is designed for up to 96 extractions of DNA fragments of 70 bp to 10 kb from TAE, TBE, standard or low-melt agarose gels, using the solubilization and binding buffer QG and the QIAquick® 96 PCR Purification Kit on a QIAvac 96 vacuum manifold.

Please be sure to read the *QIAquick Multiwell PCR Purification Handbook* and the detailed QIAquick PCR Purification Kit Protocol carefully before beginning this procedure.

Important notes before starting

- Buffer QG (Cat. No. 19063) and isopropanol (100%) are required.
- 3 M sodium acetate, pH 5.0, may be necessary.
- An oven at 50°C is required.
- This protocol requires a Square-Well Block (Cat. No. 19573) and Tape Pads (Cat. No. 19570) to simplify handling at the solubilization step. Use of other devices such as microtubes is also possible for the solubilization step.
- Add ethanol (96–100%) to Buffer PE (concentrate) before use (see bottle label for volume).
- QIAvac 96 operates with any vacuum source (e.g. a house vacuum, vacuum pump, or water aspirator) that generates negative pressure. The QIAquick 96 PCR Purification System should be used with vacuum pressures between –100 to –600 mbar (–75 to –450 mm Hg) when the vacuum is applied to an **empty** QIAquick 96 Plate on the QIAvac 96. The vacuum pressure is the differential pressure between the inside of the manifold and the atmosphere (standard atmospheric pressure: 1013 mbar, or 760 mm Hg). Use of a vacuum source that generates vacuum outside of this range, especially one that generates insufficient vacuum may reduce DNA yield and purity.
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during manipulations.
- A reservoir or multichannel pipet facilitates liquid handling at many steps in the QIAquick 96 protocol.

Procedure

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

   Minimize the size of the gel slices by removing extra agarose.

2. **Weigh the gel slices. Put each gel slice into a well of a Square-Well Block. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg ~ 100 µl) in each well. Seal the block with tape.**

   For example, add 300 µl of Buffer QG to each 100 mg of gel. For >2% agarose gels, add 6 volumes of Buffer QG. The maximum amount of agarose gel slice per well is 400 mg; use more than one well for gel slices >400 mg.

3. **Incubate the block at 50°C for 10 min (or until gel is completely dissolved) in an oven. To help dissolve gel, mix by inverting the block every 2–3 min during the incubation.**

   IMPORTANT: Solubilize the agarose gel completely. For >2% agarose gels, increase incubation time.

   During the incubation, prepare QIAvac 96:

   Place waste tray inside the QIAvac base, and place the top plate squarely over the base. Seal unused wells of QIAquick 96 Plate with tape, and place plate securely in the QIAvac top plate. Attach the QIAvac 96 to a vacuum source.

4. **After the gel slices have dissolved completely, remove the tape from the block and check the color of each mixture is yellow (similar to Buffer QG without dissolved agarose).**

   If, after solubilization of the agarose, the binding mixture appears orange or violet add 10 µl of 3 M sodium acetate, pH 5.0, to the respective samples. Seal the block with a new tape and mix by inverting the block. The color of the mixtures will turn to yellow. Remove the tape from the block.

5. **Add 1 gel volume of isopropanol to each of the samples. Seal the block with tape and mix by inverting 6–8 times. Remove the tape from the block.**

   For example, if the agarose gel slice was 200 mg, add 200 µl isopropanol.

   This step increases the yield of DNA fragments <500 bp and >4 kb. For DNA fragments from 500 bp to 4 kb addition of isopropanol has no effect on yield.

6. **Apply the samples to the wells of the QIAquick 96 Plate and switch on vacuum source. After the samples in all wells have passed through, switch off vacuum source.**

   The maximum loading volume of a well is 1 ml. For sample volumes more than 1 ml, simply load again.

7. **(Optional): Add 1 ml of Buffer QG to the wells of the plate and apply vacuum.**

   This step will remove all traces of agarose. It is only required when the eluate will be used for direct sequencing, in vitro transcription, or microinjection.

8. **To wash, add 1 ml of Buffer PE to the wells and apply vacuum.**

9. **Repeat step 8.**

10. After Buffer PE in all wells has been drawn through, apply maximum vacuum for an additional 10 min to dry the membrane.

IMPORTANT: This step removes residual Buffer PE from the membrane. The removal is only effective when maximum vacuum is used (i.e. turn off vacuum regulator valves or leakage valves if they are used), allowing maximum airflow to go through the wells.

11. Switch off vacuum source and ventilate QIAvac 96 slowly. Lift the top plate from the base (not the QIAquick Plate from the top plate), vigorously rap the top plate on a stack of absorbent paper until no further liquid comes out, and blot the nozzles of the QIAquick Plate with clean absorbent paper.

Proceed either to step 12a, or 12b, as desired.

This step removes residual Buffer PE which may be present around the outlet nozzles and collars of the QIAquick Plate. Residual ethanol, from Buffer PE, may inhibit subsequent enzymatic reactions, such as sequencing.

12a. For elution into provided collection microtubes:

Replace waste tray with the provided blue collection microtube rack containing 1.2 ml collection microtubes. Place the top plate back on base.

12b. For elution into a 96-well plate:

Replace waste tray with empty blue collection microtube rack (provided with QIAvac 96) and place a 96-well plate directly on the rack. Place the top plate back on base.

13. To elute, add 80 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or water (pH 7.0) to the center of each well of the QIAquick 96 Plate, allow to stand for 1 min, and switch on vacuum source for 5 min. Once finished, switch off vacuum source and ventilate QIAvac 96 slowly. Alternatively, for increased DNA concentration, use 60 µl elution buffer.

IMPORTANT: Ensure that the elution buffer is dispensed directly onto the center of QIAquick membrane for complete elution of bound DNA. Please note that the average eluate volume is 60 µl from 80 µl elution buffer volume, and 40 µl from 60 µl elution buffer.

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

Ordering Information

Product	Contents	Cat. No.
QIAquick 96 PCR Purification Kit		
QIAquick 96 PCR Purification Kit (4)*†	For purification of 4 x 96 PCR samples: 4 QIAquick 96 Plates, Buffers, Collection Microtubes (1.2 ml), Caps	28181
Related products		
QIAquick 8 PCR Purification Kit (10)†‡	For purification of 10 x 8 PCR samples:10 QIAquick 8 Strips, Buffers, Collection Microtubes (1.2 ml), Caps	28142
Accessories		
Buffer QG	250 ml Solubilization and Binding Buffer (with pH indicator)	19063
QIAvac 96	Vacuum manifold for processing QIAGEN 96-well plates: includes QIAvac 96 Top Plate, Base, Waste Tray, Plate Holder, Rack of Collection Microtubes (1.2 ml)	19504
Vacuum Regulator	For use with QIAvac manifolds	19530
Square-Well Block (24)	96-well blocks with 2.2 ml wells, 24 blocks per case	19573
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks, 25 sheets per pad, 5 pads per pack	19570

* *Requires use of QIAvac 96*
† *Larger kit sizes available; please inquire*
‡ *Requires use of QIAvac 6S*

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor.
Selected handbooks can be downloaded from **www.qiagen.com/literature/handbooks/default.asp**.
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from **www.qiagen.com/ts/msds.asp**