QIAquick® Gel Extraction Kit

The QIAquick Gel Extraction Kit (cat. nos. 28704 and 28706) can be stored at room temperature (15–25°C) for up to 12 months.

For more information, please refer to the *QIAquick Spin Handbook*, which can be found at: www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- This protocol is for the purification of up to 10 µg DNA (70 bp to 10 kb).
- The yellow color of Buffer QG indicates a pH ≤7.5. DNA adsorption to the membrane is only efficient at pH ≤7.5.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- Isopropanol (100%) and a heating block or water bath at 50°C are required.
- All centrifugation steps are carried out at 17,900 x *g* (13,000 rpm) in a conventional table-top microcentrifuge.
- Symbols: ● centrifuge processing; ▲ vacuum processing.

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.
2. Weigh the gel slice in a colorless tube. Add 3 volumes Buffer QG to 1 volume gel (100 mg gel ~ 100 µl). The maximum amount of gel per spin column is 400 mg. For >2% agarose gels, add 6 volumes Buffer QG.
3. Incubate at 50°C for 10 min (or until the gel slice has completely dissolved). Vortex the tube every 2–3 min to help dissolve gel. After the gel slice has dissolved completely, check that the color of the mixture is yellow (similar to Buffer QG without dissolved agarose). If the color of the mixture is orange or violet, add 10 µl 3 M sodium acetate, pH 5.0, and mix. The mixture turns yellow.

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4. Add 1 volume isopropanol to the sample and mix. 100 µl per 100 mg of original mass

5. Place a QIAquick spin column in ● a provided 2 ml collection tube or into ▲ a vacuum manifold. To bind DNA, apply the sample to the QIAquick column and ● centrifuge for 1 min or ▲ apply vacuum to the manifold until all the samples have passed through the column. ● Discard flow-through and place the QIAquick column back into the same tube. For sample volumes of >800 µl, load and spin/apply vacuum again.

(should be recorded)

6. If the DNA will subsequently be used for sequencing, in vitro transcription, or microinjection, add 500 µl Buffer QG to the QIAquick column and ● centrifuge for 1 min or ▲ apply vacuum. ● Discard flow-through and place the QIAquick column back into the same tube.

@13,000 RPM

7. To wash, add 750 µl Buffer PE to QIAquick column and ● centrifuge for 1 min or ▲ apply vacuum. ● Discard flow-through and place the QIAquick column back into the same tube.

   Note: If the DNA will be used for salt-sensitive applications (e.g., sequencing, blunt-ended ligation), let the column stand 2–5 min after addition of Buffer PE.

   Centrifuge the QIAquick column in the provided 2 ml collection tube for 1 min to remove residual wash buffer.

8. Place QIAquick column into a clean 1.5 ml microcentrifuge tube.

9. To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the QIAquick membrane and centrifuge the column for 1 min. [For increased DNA concentration, add 30 µl Buffer EB to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge for 1 min. After the addition of Buffer EB to the QIAquick membrane, increasing the incubation time to up to 4 min can increase the yield of purified DNA.]

* Do only what in between the brackets for increased DNA yield

10. If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

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