Quick-Start Protocol April 2016

MinElute® Gel Extraction Kit

The MinElute Gel Extraction Kit (cat. nos. 28604 and 28606) can be stored at room temperature (15–25°C) for up to 12 months if not otherwise stated on label. Store columns at 2–8°C upon arrival.

Further information

- *MinElute Handbook*: **www.qiagen.com/HB-2069**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for cleanup of up to 5 µg DNA fragments (70 bp to 4 kb).
- The yellow color of Buffer QG indicates a pH of ≤7.5. Adsorption of DNA to the membrane is efficient only at pH ≤7.5.
- Add ethanol (96–100%) to Buffer PE concentrate 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 tabletop microcentrifuge at room temperature (15–25°C).
- 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 of Buffer QG to 1 volume of gel (100 mg gel ~ 100 µl). The maximum amount of gel slice 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 during incubation to help dissolve the gel.

4. 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 color of the mixture will turn to yellow.
5. Add 1 gel volume of isopropanol to the sample and mix by inverting.
6. Place a MinElute spin column ● in a provided 2 ml collection tube or ▲ into a vacuum manifold. For information about set up, see the *MinElute Handbook*.
7. Apply sample to the MinElute column and ● centrifuge for 1 min or ▲ apply vacuum until the entire sample has passed through the column. ● Discard flow-through and place the MinElute column back into the same collection tube. For sample volumes of more than 800 µl, simply load and spin again.
8. Add 500 µl Buffer QG to the MinElute column and ● centrifuge for 1 min or ▲ apply vacuum. ● Discard flow-through and place the MinElute column back into the same collection tube.
9. Add 750 µl Buffer PE to MinElute column and ● centrifuge for 1 min or ▲ apply vacuum. ● Discard flow-through and place the MinElute column back into the same collection tube.

   Note: If the DNA will be used for salt-sensitive applications, such as direct sequencing and blunt-ended ligation, let the column stand 2–5 min after addition of Buffer PE.
10. Centrifuge the column in a 2 ml collection tube (provided) for 1 min. Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.
11. Place each MinElute column into a clean 1.5 ml microcentrifuge tube. To elute DNA, add 10 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the MinElute membrane. (Ensure that the elution buffer is dispensed directly onto the membrane for complete elution of bound DNA.) Let the column stand for 1 min, and then centrifuge the column for 1 min.
12. If purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA, and mix by pipetting up and down before loading the gel.

Scan QR code for handbook.