Quick-Start Protocol March 2016

QIAEX II® Gel Extraction Kit

The QIAEX II Gel Extraction Kit (cat. nos. 20021 and 20051) can be stored at room temperature (15–25°C) for up to 12 months if not otherwise stated on label.

Further information

- *QIAEX II Handbook*: **www.qiagen.com/HB-1167**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is for cleanup of DNA fragments of 40 bp to 50 kb.
- The yellow color of Buffer QX1 indicates a pH ≤7.5.
- Add ethanol (96–100%) to Buffer PE concentrate before use (see bottle label for volume).
- A heating block or water bath at 50°C is 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).
- For purification of DNA from polyacrylamide gels or aqueous solutions, see the handbook.

1. Excise the DNA band from the agarose gel with a clean, sharp scalpel. Use a 1.5 ml microfuge tube for processing up to 250 mg agarose per tube.
2. Weigh the gel slice in a colorless tube. Add Buffer QX1 according to DNA fragment size: 6 volumes for <100 bp; 3 volumes for 100 bp – 4 kb; 3 volumes with 2 volumes of water for >4 kb. Add 6 volumes of Buffer QX1 when using >2% or Metaphor agarose gels.
3. Resuspend QIAEX II by vortexing for 30 s. Add QIAEX II to the sample and mix: Use 10 µl QIAEX II for ≤2 µg DNA; 30 µl for 2–10 µg DNA; and an additional 30 µl for each additional 10 µg DNA.

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 violet, 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 at least 5 min.
5. Centrifuge the sample for 30 s and carefully remove supernatant with a pipet.
6. Wash the pellet with 500 µl 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 Buffer PE. Resuspend the pellet by vortexing.* Centrifuge the sample for 30 s and carefully remove all traces of supernatant with a pipet. This step removes residual salt contaminants.
8. Air-dry the pellet for 10–15 min or until the pellet becomes white. If 30 µl QIAEX II suspension is used, air-dry the pellet for approximately 30 min. Do not vacuum dry, as overdrying, may lead to decreased elution efficiency.
9. To elute DNA, add 20 µl of 10 mM Tris·Cl, pH 8.5, TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0) or water and resuspend the pellet by vortexing.* Incubate according to the DNA fragment size: 5 min at room temperature (15–25°C) for ≤4 kb; 5 min at 50°C for 4–10 kb; or 10 min at 50°C for >10 kb.
10. Centrifuge for 30 s, and 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%.

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

Scan QR code for handbook.