QIAquick® Spin Handbook

QIAquick PCR Purification Kit
For purification of PCR products, 100 bp to 10 kb

QIAquick Nucleotide Removal Kit
For oligonucleotide (17-40mers) and DNA
(40 bp to 10 kb) cleanup from enzymatic reactions

QIAquick Gel Extraction Kit
For gel extraction or cleanup of DNA
(70 bp to 10 kb) from enzymatic reactions
QIAquick PCR Purification Kit Protocol using a microcentrifuge

This protocol is designed to purify single- or double-stranded DNA fragments from PCR and other enzymatic reactions (see page 8). For cleanup of other enzymatic reactions, follow the protocol as described for PCR samples or use the MinElute Reaction Cleanup Kit. Fragments ranging from 100 bp to 10 kb are purified from primers, nucleotides, polymerases, and salts using QIAquick spin columns in a microcentrifuge.

Important points before starting
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are carried out at 17,900 x g (13,000 rpm) in a conventional tabletop microcentrifuge at room temperature.
- Add 1:250 volume pH indicator I to Buffer PB (i.e., add 120 µl pH indicator I to 30 ml Buffer PB or add 600 µl pH indicator I to 150 ml Buffer PB). The yellow color of Buffer PB with pH indicator I indicates a pH of ≤7.5.
- Add pH indicator I to entire buffer contents. Do not add pH indicator I to buffer aliquots.
- If the purified PCR product is to be used in sensitive microarray applications, it may be beneficial to use Buffer PB without the addition of pH indicator I.

Procedure
1. Add 5 volumes of Buffer PB to 1 volume of the PCR sample and mix. It is not necessary to remove mineral oil or kerosene. For example, add 500 µl of Buffer PB to 100 µl PCR sample (not including oil).
2. If pH indicator I has been added to Buffer PB, check that the color of the mixture is yellow.
   If the color of the mixture is orange or violet, add 10 µl of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.
3. Place a QIAquick spin column in a provided 2 ml collection tube.
4. To bind DNA, apply the sample to the QIAquick column and centrifuge for 30–60 s.
5. Discard flow-through. Place the QIAquick column back into the same tube. Collection tubes are re-used to reduce plastic waste.
6. To wash, add 0.75 ml Buffer PE to the QIAquick column and centrifuge for 30–60 s.
7. Discard flow-through and place the QIAquick column back in the same tube. Centrifuge the column for an additional 1 min.
   IMPORTANT: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.
8. Place QIAquick column in 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 (pH 7.0–8.5) to the center of the QIAquick membrane and centrifuge the column for 1 min. Alternatively, for increased DNA concentration, add 30 µl elution buffer to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge.

**IMPORTANT**: Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA. The average eluate volume is 48 µl from 50 µl elution buffer volume, and 28 µl from 30 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, 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. 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.

Loading dye contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time. Refer to Table 2 (page 15) to identify the dyes according to migration distance and agarose gel percentage and type.
QIAquick Gel Extraction Kit Protocol

using a microcentrifuge

This protocol is designed to extract and purify DNA of 70 bp to 10 kb from standard or low-melt agarose gels in TAE or TBE buffer. Up to 400 mg agarose can be processed per spin column. This kit can also be used for DNA cleanup from enzymatic reactions (see page 8). For DNA cleanup from enzymatic reactions using this protocol, add 3 volumes of Buffer QG and 1 volume of isopropanol to the reaction, mix, and proceed with step 6 of the protocol. Alternatively, use the MinElute Reaction Cleanup Kit.

Important points before starting

- The yellow color of Buffer QG indicates a pH ≤ 7.5.
- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are carried out at 17,900 x g (13,000 rpm) in a conventional table-top microcentrifuge at room temperature.

Procedure

1. **Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.**
   Minimize the size of the gel slice by removing extra agarose.

2. **Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer QG to 1 volume of gel (100 mg ~ 100 µl).**
   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 gel slice per QIAquick column is 400 mg; for gel slices >400 mg use more than one QIAquick column.

3. **Incubate at 50°C for 10 min (or until the gel slice has completely dissolved). To help dissolve gel, mix by vortexing the tube every 2–3 min during the incubation.**
   **IMPORTANT:** Solubilize agarose completely. For >2% gels, increase incubation time.

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 of 3 M sodium acetate, pH 5.0, and mix. The color of the mixture will turn to yellow.

   The adsorption of DNA to the QIAquick membrane is efficient only at pH ≤ 7.5. Buffer QG 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. **Add 1 gel volume of isopropanol to the sample and mix.**
   For example, if the agarose gel slice is 100 mg, add 100 µl isopropanol. This step increases the yield of DNA fragments <500 bp and >4 kb. For DNA fragments between 500 bp and 4 kb, addition of isopropanol has no effect on yield. Do not centrifuge the sample at this stage.
6. Place a QIAquick spin column in a provided 2 ml collection tube.

7. To bind DNA, apply the sample to the QIAquick column, and centrifuge for 1 min.
   The maximum volume of the column reservoir is 800 µl. For sample volumes of more
   than 800 µl, simply load and spin again.

8. Discard flow-through and place QIAquick column back in the same collection tube.
   Collection tubes are reused to reduce plastic waste.

9. Recommended: Add 0.5 ml of Buffer QG to QIAquick column and centrifuge for 1 min.
   This step will remove all traces of agarose. It is only required when the DNA will
   subsequently be used for direct sequencing, in vitro transcription, or microinjection.

10. To wash, add 0.75 ml of Buffer PE to QIAquick column and centrifuge for 1 min.
    Note: If the DNA will be used for salt-sensitive applications, such as blunt-end ligation
    and direct sequencing, let the column stand 2–5 min after addition of Buffer PE,
    before centrifuging.

11. Discard the flow-through and centrifuge the QIAquick column for an additional 1 min
    at 17,900 x g (13,000 rpm).
    IMPORTANT: Residual ethanol from Buffer PE will not be completely removed unless
    the flow-through is discarded before this additional centrifugation.

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

13. To elute DNA, add 50 µl of Buffer EB (10 mM Tris-Cl, pH 8.5) or water (pH 7.0–8.5) to the
    center of the QIAquick membrane and centrifuge the column for 1 min. Alternatively,
    for increased DNA concentration, add 30 µl elution buffer to the center of the QIAquick
    membrane, let the column stand for 1 min, and then centrifuge for 1 min.
    IMPORTANT: Ensure that the elution buffer is dispensed directly onto the QIAquick
    membrane for complete elution of bound DNA. The average eluate volume is 48 µl
    from 50 µl elution buffer volume, and 28 µl from 30 µl.

    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, 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 (10 mM Tris-Cl, 1 mM
    EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

14. 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.

    Loading dye contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange
    G) that facilitate estimation of DNA migration distance and optimization of agarose
    gel run time. Refer to Table 2 (page 15) to identify the dyes according to migration
    distance and agarose gel percentage and type.