

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 $17,900 \times g$ (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 μ l 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 μ l 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 μ l from 50 μ l elution buffer volume, and 28 μ l from 30 μ l 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.