

QIAGEN Supplementary Protocol:

Cleanup of nonradioactive samples using the QIAquick® Nucleotide Removal Kit

QIAquick® Spin Columns can now be used on any vacuum manifold with luer connectors, for example, the QIAvac 6S or QIAvac 24 with QIAvac Luer Adapters. This protocol is designed for removal of primers <10 bases, enzymes, salts, and unincorporated nucleotides from biotin-, or DIG-labeled DNA fragments and oligonucleotides >17 nucleotides.

Please be sure to read the *QIAquick Spin Handbook* and the detailed QIAquick Nucleotide Removal Kit Protocol carefully before beginning the procedure.

We do not recommend the processing of radioactive samples using a vacuum manifold.

Important notes before starting

- Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
- All centrifugation steps are at $\geq 10,000 \times g$ (~13,000 rpm) in a conventional tabletop microcentrifuge.

Procedure

1. Add 10 volumes of Buffer PN to 1 volume of the reaction sample and mix.

For example, add 500 μl Buffer PN to a 50 μl reaction sample. For DNA fragments ≥ 100 bp, only 5 volumes of Buffer PN are required.

2. Prepare the vacuum manifold and QIAquick Spin Columns.

QIAvac 6S manifold: Open QIAvac 6S lid. Place QIAvac Luer Adapter(s), or blanks to seal unused slots, into the slots of QIAvac top plate, and close the QIAvac 6S lid. Place the waste tray inside the QIAvac base, and place the top plate squarely over the base. Attach the QIAvac 6S to a vacuum source. Insert each QIAquick Spin Column into a luer connector on the Luer Adapter(s) in the manifold. Seal unused luer connectors with plugs provided with the QIAvac Luer Adapter Set.

QIAvac 24 manifold: Place the QIAvac 24 lid on top of the QIAvac 24 base. Make sure that the gasket fits tightly in the groove of the QIAvac 24 lid. Insert up to 24 QIAquick Spin Columns into the luer extensions of the QIAvac 24. Close unused positions with luer caps and connect QIAvac 24 to a vacuum source.

Other vacuum manifolds: follow the supplier's instructions. Insert each QIAquick Spin Column into a luer connector.

3. To bind DNA, load the samples into the QIAquick Spin Columns by decanting or pipetting, and apply vacuum. After the samples have passed through the column, switch off the vacuum source.

4. Wash QIAquick Spin Columns by adding 750 μl of Buffer PE to each column and apply vacuum.

5. **Transfer each QIAquick Spin Column to a microcentrifuge tube or the provided 2 ml collection tubes. Centrifuge for 1 min at $\geq 15,000 \times g$ (~13,000 rpm).**
IMPORTANT: This extra spin is necessary to remove residual Buffer PE.
6. **Place each QIAquick Spin Column into a clean 1.5 ml microcentrifuge tube.**
7. **To elute DNA, add 100–200 μ l of Buffer EB (10 mM Tris-Cl, pH 8.5) or water to the column and centrifuge for 1 min. Alternatively, for increased DNA concentration, add 30–50 μ l elution buffer to the center of the QIAquick Spin Column, allow to 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.

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.

Ordering Information

Product	Contents	Cat. No.
QIAquick Nucleotide Removal Kit (50)	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28304
QIAquick Nucleotide Removal Kit (250)	250 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28306
Buffer PN	500 ml Binding Buffer	19071
QIAvac 24	Vacuum manifold for processing 1–24 spin columns: includes QIAvac 24 Base, Lid, Luer Caps	19403
QIAvac 6S	Vacuum manifold for processing 1–24 QIAGEN spin columns or 1–6 QIAGEN 8-well strips: includes QIAvac 6S Top Plate with flip-up lid, Base, Waste Tray, Blanks, Strip Holder, Rack of Collection Microtubes (1.2 ml)	19503
QIAvac Luer Adapter Set*	For processing 1–24 QIAGEN spin columns on QIAvac 6S: 6 adapters with 4 luer connectors each, 24 plugs	19541
Vacuum Regulator	For use with QIAvac manifolds	19530

* Compatible only with QIAvac Top Plates containing flip-up lid.

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor. Selected handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.asp. Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp.

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