User-developed protocol

User-Developed Protocol:

Forensic post-PCR purification protocol using the MinElute® PCR Purification Kit

This protocol, adapted from Smith and Ballantyne (2007), is for forensic post-PCR purification using the MinElute PCR Purification Kit.* It is designed to purify double-stranded DNA fragments from PCR reactions resulting in high end-concentrations of DNA. Fragments ranging from 70 bp to 4 kb are purified from primers, nucleotides, polymerases, and salts using MinElute spin columns in a microcentrifuge.

Note: Do not add pH indicator to Buffer PB for this protocol. Buffer PB (cat. no.19066) can also be purchased separately.

Important: Please read the *MinElute Handbook*, paying careful attention to the "Safety Information" and "Important Notes" sections, before beginning this procedure.

Important: The MinElute PCR Purification Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents to be supplied by the user

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- Ethanol (96–100%)
- Pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- Microcentrifuge

Important points before starting

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

* Smith, P.J. and Ballantyne, J. (2007) Simplified low-copy-number DNA analysis by post-PCR purification. J. Forensic Sci. **52**, 820

Procedure

1. **Add 5 volumes of Buffer PB to 1 volume of the PCR sample and mix.**
   For example, add 250 µl Buffer PB to 50 µl PCR sample.
2. **Place a MinElute column in a 2 ml collection tube (provided).**
3. **To bind DNA, apply the sample to the MinElute column and centrifuge from 1 min.**
   For maximum recovery, transfer all traces of sample to the column.
4. **Discard flow-through. Place the MinElute column back into the same tube.**
5. **To wash, add 700 µl Buffer PE to the MinElute column and centrifuge 1 min.**
6. **Discard flow-through and place the MinElute column back to the same tube.**
7. **Repeat the steps 5 and 6 three times to make a total of four washes.**
8. **Discard flow-through and place the MinElute column back in the same tube. Centrifuge the column for an additional 1 min at maximum speed.**
   Important: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.
9. **To elute DNA, add 10 µl Buffer EB (10 mM Tris·Cl, pH 8.5) to the center of the membrane, let the column stand for 1 min, and then centrifuge for 1 min.**
   Important: Ensure that Buffer EB is dispensed directly onto the center of the membrane for complete elution of bound DNA. The average eluate volume is 9 µl from 10 µl elution buffer volume.

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

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