

## **User-Developed Protocol:**

### **Forensic post-PCR purification protocol using the MinElute<sup>®</sup> 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:** This protocol uses Buffer PB rather than Buffer PBI which is supplied with the MinElute PCR Purification Kit. Buffer PB is available separately (cat. no. 19066).

**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  $\geq 10,000 \times g$  (~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 for 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.

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