User-Developed Protocol

DNA extraction from fired and unfired ammunition using the EZ1® DNA Investigator® Kit

This protocol has been adapted by the San Diego Police Department (SDPD) Crime Laboratory from the standard QIAGEN pretreatment for casework samples, and is intended for the lysis and extraction of DNA from forensic samples of fired (casings) and unfired (cartridges) ammunition.

Details of the modified protocol can be found in the following publication:

Montpetit, S., O'Donnell, P. (2015) An optimized procedure for obtaining DNA from fired and unfired ammunition. Forensic Science International: Genetics **17**, 70–74.

This protocol has not been thoroughly tested and optimized by QIAGEN.

IMPORTANT: Please read the "Safety Information" and "Important Notes" sections in the *EZ1 DNA Investigator Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate Safety Data Sheets (SDSs), available from the product supplier. The EZ1 DNA Investigator Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Important points before starting

- If using the EZ1 DNA Investigator Kit for the first time, read "Important Notes" in the *EZ1 DNA Investigator Handbook*.
- Diluted Buffer ATL, which may be used as a lysis buffer, is not included in the EZ1 DNA Investigator Kit. Buffer ATL (cat. no. 19076) should be purchased separately.

Optional alternative lysis buffer:

Prepare diluted ATL: one (1) part Buffer ATL, two (2) parts TE-4

e.g., 10 ml Buffer ATL

20 ml TE-4 Buffer (10 mM Tris, 0.1 mM EDTA; pH 8.0)

Things to do before starting

- Heat a thermomixer or incubator to 56°C for the proteinase K digestion in step 7.

Equipment and reagents to be supplied by user

For all users

- Lysis Buffer (10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 2% SDS) **(1)**
- Buffer ATL (QIAGEN cat. no. 19076) and TE-4 (10 mM Tris, 0.1 mM EDTA; pH 8.0) – **optional alternative lysis buffer**
- Proteinase K (2 ml QIAGEN cat. no. 19131, or 10 ml QIAGEN cat. no. 19133)
- 5 ml tube; graduated, flat-base (QIAGEN cat. no. 990552)
- Pipet tips (pipet tips with aerosol barriers to prevent cross-contamination are recommended)
- 10–200 µl and 200–1000 µl pipettor

- Centrifuge
- Cotton swabs
- Thermomixer or alternative incubator

For EZ1 Advanced XL users

- EZ1 Advanced XL instrument (QIAGEN cat. no. 9001492)
- EZ1 DNA Investigator Kit (QIAGEN cat. no. 952034)
- EZ1 Advanced XL DNA Investigator Card (QIAGEN EU cat. no. 9018699, QIAGEN NA cat. no. 9019933)

Procedure

Digestion

1. Place the individual cartridge or casing upright in a sterile 5 ml screw cap tube.
2. Add 850 µl lysis buffer* and 34 µl of proteinase K (1 µl carrier RNA optional).
   Note: Casing will be almost submerged (see Figure 1).
   *(QIAGEN alternative equivalent lysis buffer is diluted ATL, not tested by SDPD).

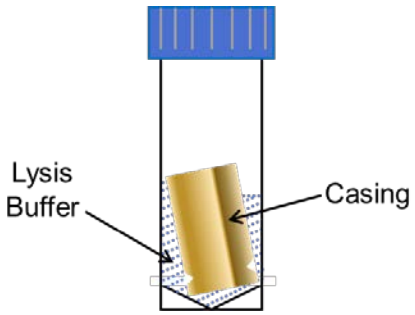

Figure 1. Positioning of casing in 5 ml tube with lysis buffer.

3. Incubate at 56°C for 30 min.
4. Remove the casing or cartridge from the lysis buffer.
5. Using a cotton swab, remove any residual buffer and biological material from the casing/cartridge.
6. After swabbing the casing/cartridge, return the head of the cotton swab to the same 5 ml tube containing lysis buffer.
7. Incubate at 56°C for an additional 90 min.

DNA purification

8. Remove the cotton swab head and transfer the sample lysate (~884 µl) to a 2 ml EZ1 sample tube.
9. Place the 2 ml tube directly in the appropriate sample position in the EZ1 sample rack (Do not add buffer MTL).

10. Continue with EZ1 DNA Investigator Kit Protocol: DNA Purification (Large-Volume Protocol).

QIAGEN recommends starting the EZ1 purification protocol while the lysate is warm.

Reference

1. Montpetit, S., O'Donnell, P. (2015) An optimized procedure for obtaining DNA from fired and unfired ammunition. Forensic Science International: Genetics **17,** 70–74.