DIFFERENTIAL EXTRACTION ON THE EZ1® ADVANCED XL

Background

The EZ1® Advanced XL and the EZ1® DNA Investigator Kit reproducibly automate purification of genomic DNA from 1-14 samples encountered in forensic applications. Purification is efficient, and purified DNA is suitable for use in downstream analyses, such as quantitative PCR and STR analysis, with high signal-to-noise ratios.

The magnetic-particle chemistry is similar to that currently used at the Marshall University Forensic Science Center (MUFSC) in Promega's DNA IQ method, and can provide a higher yield when Carrier RNA, included in the kit, is utilized. The EZ1 Advanced XL performs all steps of DNA purification, post-digestion, and can accommodate an input volume of 250µL for differential extraction.

Magnetic-particle technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles. DNA is isolated from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet. The DNA is then efficiently washed and eluted in water or TE buffer.

Summary of Procedure

This protocol is for the differential DNA extraction of samples containing a mixture of spermatozoa (sperm fraction) and non-sperm cells (epithelial fraction). Samples are exposed to relatively weak digestion conditions to lyse non-sperm cells, leaving spermatozoa intact. Centrifugation pellets the spermatozoa and allows the epithelial fraction lysate to be removed from the mixture. Stronger digestion conditions, including the addition of dithiothreitol (DTT), will lyse spermatozoa. Both fractions are then purified and concentrated on the EZ1® Advanced XL instrument. Following this protocol, up to 14 samples (producing 14 sperm fractions and 14 epithelial fractions) can be extracted concurrently.

Sample Handling

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- **Safety Manual**, *Universal Precautions*
- **Quality Assurance Manual**, *General Sample Control and Forensic Sample Preservation Policy*
- **Analytical Procedures Manual**, *Forensic Evidence Handling*

After extraction is complete, samples are securely stored at 2-8C.

Warnings and Precautions

Buffer G2 contains chaotropic salt, which is an irritant. Take appropriate laboratory safety measures and wear gloves when handling. This reagent is **not** compatible with disinfecting agents which contain bleach.

Reagent cartridges contain ethanol, isopropanol and guanidine thiocyanate. These substances should be considered flammable, harmful, and irritants.

Reagents and Materials

EZ1® Advanced XL instrument
EZ1® DNA Investigator Kit (48 reactions)
    48 Reagent Cartridges
    50 Tip Holders

50 Filter-Tips
50 2mL Sample Tubes (Digestion)
50 1.5mL Elution Tubes
11mL Buffer G2
2x250µL Proteinase K
310µg Carrier RNA
Additional Buffer G2 (260mL bottle, available separately from the EZ1® DNA Investigator Kit)
1M Dithiothreitol (DTT)
Pipettes (1000µL, 200µL, 40µL, and 10µL)
Pipette tips
Heat block
1.7mL or 2mL micro-centrifuge tubes
Spin baskets compatible with micro-centrifuge tubes
Permanent marker
Kimwipes
Vortex
Centrifuge
Protective eyewear
Gloves
Lab coat

Reagents and Materials – Storage and Handling

All reagents and materials are to be kept under sterile conditions. Store all reagents according to the manufacturers' recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. Record in the **Reagent Log**.

Quality Control

Use of the **EZ1® Differential Workbook** is required for documentation. All information must be completed.

Positive Control

Extraction Positive Control (TF- Optional): Positive control(s) are processed and run with each set of extractions and used to ensure the extraction, amplification and typing procedures are working as expected.

Negative Control

Extraction Negative Control: Reagent negative control(s) are processed and run with each set of extractions and consists of all reagents used in the procedure but contains no DNA sample.

Procedure

When a new kit is opened:

- Tip Holders, Filter-Tips, Sample Tubes and Elution Tubes must be cross-linked at 6J/cm^2.
- 310µL of nuclease-free water must be added to the dehydrated carrier RNA. Dispense carrier RNA into 30µL aliquots, and store frozen.

Before starting:

- Pre-warm one heat block to 56°C and another to 70°C.
- Allow Carrier RNA aliquot to equilibrate to room temperature.

Separation & Digestion:

1. Label all micro-centrifuge tubes, EZ1 sample tubes and EZ1 elution tubes with permanent marker or printed labels.
2. Place a sampled portion of the evidence into a 1.7mL or 2mL micro-centrifuge tube.
3. Add 190µL - 250uL of Buffer G2 to each sample. Volume cannot exceed 250uL for the EZ1 extraction described in this protocol.
4. Add 10µL of Proteinase K. Vortex for 10 seconds.
5. Incubate at 56°C for 1-2 hours. Centrifuge briefly.

   NOTE: The manufacturer has indicated that incubating more than 2 hours could result in loss of sample yield.
6. Transfer the substrate and liquid to a micro-centrifuge tube containing a spin basket. Centrifuge at 13,200 rpm for 5 min.
7. Transfer supernatant (i.e. the epithelial fraction) to a labeled EZ1 extraction tube being careful not to disrupt the sperm pellet.
8. Add 1µL of carrier RNA solution to each epithelial cell lysate. **EZ1 purification can now be initiated on the epithelial fraction (steps 15-27).**
9. Add 500uL Buffer G2 to the sperm pellet, vortex and spin at 13,200 rpm for 5 min. Remove and discard supernatant, being careful not to disrupt the sperm pellet.
10. Repeat step 9 at least two times for a total of three washes.

    NOTE: Greater than three washes may be performed at the analyst's discretion. This is sometimes necessary if the sample contains a high concentration of non-sperm cells.
11. Add 160µl Buffer G2 to the sperm pellet, vortex and spin down briefly.
12. Add 10µl Proteinase K, 40µl of 1M DTT, and 1µl carrier RNA. Vortex vigorously for 10 seconds.
13. Incubate at 70°C for 10 minutes. Vortex for 10 seconds every minute during this incubation (i.e. place samples in the 70°C heat block; start a timer for 10 minutes; after 60 seconds, remove samples from heat block and pause the timer; vortex samples vigorously for 10 seconds; return samples to heat block and resume the timer; repeat every 60 seconds).
14. Transfer sperm fraction to a labeled EZ1 extraction tube. **EZ1 purification can now be initiated on the sperm fraction (steps 15-27).**

EZ1 Purification:

15. Switch on the EZ1 workstation.

16. Press “START” to start the protocol setup.

17. Choose the appropriate protocol.
    - Press “1” for Trace Protocol (for volumes up to 200uL and substrate removed)
    - Press “3” for Large-Volume Protocol (for volumes up to 500uL and substrate removed)

18. Choose water or TE as the elution buffer and a 40uL elution volume. Press any key to start the worktable setup.

19. Open the workstation door and invert 1-14 reagent cartridges at least twice to mix the magnetic particles. Tap cartridges to deposit reagents at the bottom of the well. Load reagent cartridges into the cartridge rack, (Make sure they click in place.)

20. Load 1-14 opened elution tubes (labeled) into the first row of the tip rack.

21. Load 1-14 tip holders containing filter-tips into the second row of the tip rack.

22. Load 1-14 opened sample tubes containing digested samples into the back row of the tip rack.

23. Close the workstation door and press “START” to begin the purification procedure.

24. “Protocol Finished” should display when it is finished.

25. Open the workstation door and remove the elution tubes containing purified DNA. Replace tube caps and store samples appropriately.

26. Remove spent cartridges, tip holders, filter-tips and samples tubes and discard appropriately.

27. Close the workstation door and follow the prompts to UV irradiate the workstation for 30 minutes.

Comments on Storage

Non-amplified DNA must be stored separately from amplified product. Store samples at 2-8°C.

Technical Assistance

For information and assistance regarding the performance or applications, contact the Qiagen Technical Service Department 1-800-362-7737.

Reference

1. EZ1® Investigator Handbook, Fourth Edition, April 2009. Qiagen