# DNA EXTRACTION ON THE EZ1® ADVANCED XL - Reference Samples

## *Background*

The EZ1® Advanced XL and the EZ1® DNA Investigator Kit reproducibly automates 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 technology is similar to that currently used at the Marshall University Forensic Science Center (MUFSC) in Promega's DNA IQ, and can provide a higher yield when the Carrier RNA, included in the kit, is utilized. The EZ1 Advanced XL performs all steps of the sample extraction, post-digestion, and can accommodate an input volume of 200µL for reference sample extractions.

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 100µL water or TE buffer, per the MUFSC validation[1].

## *Summary of Procedure*

The reference extraction must be performed using blood card punches (1-3), swab cuttings, or whole swabs with the Trace Protocol or Trace Tip Dance Protocol as outlined in the Qiagen EZ1® Extraction- Reference Samples worksheet. The EZ1® Advanced XL is utilized in conjunction with EZ1® DNA Investigator Kit available from Qiagen, which includes all required reagents and consumables with the exception of nuclease free water to perform 48 extractions.

## *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*

Lysis buffer 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® 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

Pipettes (1000µL, 200µL, 40µL, and 1µL)
Universal pipette tips
Heat Block
Spin Baskets
2mL Dolphin Tubes
Gloves
Lab Coat
Permanent Marker
Kimwipes
Vortex
Centrifuge
Protective Eyewear

## *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 **Qiagen EZ1® Extraction- Reference Samples 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, aliquotted (20µL), and frozen.

**Before starting:**

- Pre-warm the heat block to 56C prior to beginning the procedure.
- Allow Carrier RNA aliquot to equilibrate to room temperature.
- Prepare a G2 Buffer dilution with deionized water at a 1:1 ratio for n+1 samples.

1. Label sample tubes and elution tubes with permanent marker.

2. Add substrate to sample tube. *Entire swab, swab cutting, 1-3 FTA blood card punches. Using an entire swab or large substrate will not work using the Tip Dance Protocol. If substrate is large (e.g. whole swab or cutting greater than ~1cmX1cm) use a spin basket.*

   **NOTE:** If the substrate will be retained, the digestion should be performed using 2mL Dolphin tubes.

3. Manually add 290µL diluted G2 buffer to each sample tube.

4. Manually add 10µL Proteinase K to each sample tube. Vortex 10 seconds.

5. Incubate on Thermomixer 56°C for 15 minutes at 900 RPM. Vortex and spin down briefly.

6. Remove substrate from sample tube OR leave sample in tube if using Tip Dance Protocol. If removing substrate, a witness must observe this transfer and initial the appropriate space on the worksheet.

   **Note: Optional step to dry substrate for maximum yield: Place substrate into Spin basket. Centrifuge at 13200rpm for 5 minutes. Remove Spin basket and place in a new labeled sample tube for storage. Transfer entire lysate to labeled sample tube for extraction.**

7. Add 1µL Carrier RNA to each sample tube containing lysate.

   **NOTE:** Optional step. Extraction may be performed without RNA per validation.

8. Switch on EZ1 Workstation. Press “START” to begin the protocol setup.

9. Choose the appropriate protocol.
   - Press “1” for Trace Protocol (for volumes up to 200uL and substrate removed)
   - Press “2” for Trace Tip Dance Protocol (if not removing the substrate)
   - Press “3” for Large-Volume Protocol (for volumes up to 500uL and substrate removed)

10. Choose the elution buffer by pressing “1” for water or “2” for TE buffer.

11. Press “3” for 100µL elution volume. Press any key to begin worktable setup.

12. Open the workstation door and invert the reagent cartridges twice to mix contents. Tap the cartridges to deposit the reagents at the bottom of the well.

13. Place appropriate number of cartridges into the cartridge rack with the labeled tab facing out. Ensure that the cartridges click into place.

14. Place the appropriate number of open elution tubes into the first row of the tip rack. A witness must check the setup of the elution tubes against the worksheet and initial the appropriate space on the worksheet

15. Place the appropriate number of tip holders containing filter-tips into the second row of the tip rack.

16. Load the opened sample tubes containing digested samples into the fourth row of the tip rack. A witness must check the setup of the sample tubes against the worksheet and initial the appropriate space on the worksheet.

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

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

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

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

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

## *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