Intermountain Forensics	SOP #	EXT-200
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Non-Differential EZ1 DNA investigator Extraction

1. Purpose

This document describes the procedures for performing a Non-differential digest and extraction on the Qiagen EZ1 Advanced XL (EZ1) instrument.

2. Summary

Describes pre-processing reagent preparation. Materials and protocol for non-differential extractions is provided. This protocol is for extraction using the Qiagen EZ1 Advanced Extraction instrument with the Qiagen DNA Investigator Extraction Kit.

3. Procedure

Prepare Reagents

1. Diluted ATL
    a. Mix one-part ATL Buffer with two-parts TE to create a diluted ATL working solution (dATL)
        i. **Recommended:** Transfer 15mL ATL into 30mL TE in a 50ml conical tube as needed to meet processing volume requirement
    b. Store at room temperature.
2. Carrier RNA
    a. Add 310µL dH2O or TE to the vial of carrier RNA (310µg), provided in the EZ1 DNA Investigator kit.
        i. **Recommended:** Transfer entire volume into 20ul aliquots
    b. Aliquots should be stored frozen
3. DTT
    a. Dissolve 1.55g DTT powder into 10mL of water.
        i. **Recommended:** Transfer entire volume into 200ul aliquots
    b. Aliquots should be stored frozen.

Non-Differential Digest

4. Warm MTL buffer at 70º C.
5. Create a master mix using 480µL dATL Buffer and 20µL Proteinase K, and 1µL cRNA for each sample/reagent blank, plus overage, and mix thoroughly by vortexing. Add 500µL to each sample and reagent blank.
    a. **Note**: For hair roots and samples that may contain semen that are not being processed as a differential, add 40µL of DTT.
6. Incubate at 56°C for 1-16 hours in a thermomixer shaking at 900 rpm.
7. If Lyse and Spin Basket kit is used, centrifuge at max speed for 1 minute and discard the spin basket. Remove the substrate by transferring supernatant to a new labeled tube if no basket is used.
    a. **Note:** Lyse and Spin Basket kit should be used whenever possible. Other sampling tubes can be substituted in rare circumstances where Lyse and Spin is not feasible.
8. Add 400µL MTL buffer to each tube and vortex thoroughly. The tubes are ready for the EZ1 instrument.

    Note: Starting the EZ1 run with the samples still warm will help avoid possible precipitation.

4. References

EZ1® Advanced XL User Manual
EZ1® DNA Investigator® Handbook
Investigator® Lyse&Spin Basket Kit Handbook

5. Definitions

dATL: Working solution of Qiagen ATL buffer that is created from the stock ATL reagent