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 digestion and extraction on the Qiagen EZ1 Advanced XL (EZ1) instrument.

2. Summary

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

3. Procedure

Reagent Preparation

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 dH_2O or TE to the vial of carrier RNA (310µg), provided in the EZ1 DNA Investigator kit.
    b. Transfer entire volume into 20ul aliquots
    c. Aliquots should be stored frozen
3. DTT
    a. Dissolve 1.55g DTT powder into 10mL of water.
    b. Transfer entire volume into 200ul aliquots
    c. Store the aliquots frozen.

Documentation

1. Open a DOC-307 Case Chronicle document and save a copy to the Batches in Progress folder on the Shared Drive as the Batch ID with the following naming format: YYMMDDAnalyst Initials (Example: 230215SW)
    a. If previous extractions were performed for this batch, then open the previously saved document.
2. Click on the Extraction EZ1 ND tab of the saved Case Chronicle.
3. Enter the following information into the header of the document:
    a. Batch ID (YYMMDDAnalyst Initials)
    b. Performed by
    c. Date of extraction

d. Reagent Lot Numbers

4. Enter the sample identification numbers for all samples that will be extracted in the extraction.
    a. Create a separate Extraction EZ1 ND tab for each separate extraction set that is completed. Note: If unknown samples and reference samples are being extracted, then there must be at least 2 separate extractions and associated documentation tabs.
5. For forensic case samples, enter a reagent blank named with the following name convention: Numerical portion of the IMF Case Number without the place holder zeroes – Batch ID – RB or RBR (Example: 231-230215SW-RB) between each set of case samples included on the tray.
    a. RBR is to be used for reagent blanks associated with reference sample extractions.

Item ID		Final Extraction Volume
IMF-23-00001-01-01-A		40
IMF-23-00001-02-01-A		40
231-230215SW-RB		40
IMF-23-00002-01-01-A		40
232-230215SW-RB		40

6. For Keepsake case samples, enter two consecutive reagent blanks at the end of the list of samples, named with the following name convention: RB-batch ID number – END1 or END2 (Example: RB-230215SW-END1 and RB-230215SW-END2)

Item ID		Final Extraction Volume
IMF-23-00001-01-01-A		40
IMF-23-00001-02-01-A		40
RB-230215SW-END1		40
RB-230215SW-END2		40

Non-Differential Digest

1. Warm MTL buffer at 70º C.
2. Create a master mix by combining 480µL dATL Buffer and 20µL Proteinase K, and 1µL cRNA for each sample/reagent blank, plus overage, and mix thoroughly by vortexing.
    a. Refer to the header of DOC-307 Case Chronicle for the quantities of master mix required for the batch.

3. Opening each tube individually, pipette 500µL of the master mix to each tube containing sample.
4. For hair roots and samples that may contain semen that are not being processed as a differential, add 40µL of DTT to each tube.
5. After adding master mix to all samples associated with a forensic case, create one reagent blank by pipetting 500µL of the master mix into an empty tube between cases.
    a. If DTT was added to a sample in the case, also add 40µL of DTT.
    b. If the extraction batch is comprised of only Keepsake samples, reagent blanks may be limited to only 2 at the end of the entire extraction set.
6. Incubate the tubes at 56°C for a minimum of 1 hour in a thermomixer shaking at 900 rpm.
    a. The incubation time period may be extended to as long as 16 hours, depending on the nature of the sample and previous analysis results, if available.
7. Centrifuge the tubes at max speed for 1 minute and discard the spin basket.
    a. Lyse and Spin Basket kit should be used whenever possible.
    b. If another type of sampling tube is substituted in rare circumstances where Lyse and Spin is not feasible, the sample must be transferred to a spin basket using decontaminated tools prior to spinning.
8. Discard the substrate and the spin basket.
9. Add 400µL MTL buffer to each tube, close the tube, and vortex thoroughly.
    a. **Note**: Starting the EZ1 run with the samples still warm will help avoid possible precipitation.

EZ1 Instrument Run for Lysate Purification

1. Ensure the EZ1 Advanced XL DNA Investigator Flip-Cap Card is loaded in the instrument.
2. Turn the instrument on by clicking the toggle switch on the back panel of the instrument, next to the power cable.
3. Open the EZ1 door, press **START** on the control panel.
4. Set up the EZ1 instrument worktable:
    a. Invert reagent cartridges to mix the magnetic particles then tap the cartridges to deposit the reagents at the bottom of their wells and check that the magnetic particles are completely resuspended.
    b. Load the reagent cartridges into the cartridge rack by sliding the reagent cartridge into the rack and then pressing down until it clicks into place.
    c. Load opened elution tubes into the first row of the tip rack.
        i. Prior to loading tubes, mark each with the associated item number that will be eluted into the tube.
    d. Load tip holders and filter-tips into the second row of the tip rack.
    e. Load open sample tubes into the third row of the tip rack.

i. Cutting off the lids can lower contamination risk if flip cap tubes are used.

4. Select the following parameters for the instrument run:
    a. Press “3” for the “Large Volume Protocol”
    b. Press “1” to elute in water
    c. Press “1” to for a 40 µL elution volume
5. Press **Enter** to move through the instrument set-up verification prompts.
    a. Review the set-up of the instrument to confirm it matches the prompts.
6. Close the EZ1 door.
7. Press **START** to start the protocol run.
8. After the run is finished, remove and cap the elution tubes, which contain the purified samples, and discard the sample-preparation waste and used cartridges.
    a. Do not place the waste in a receptacle which also may contain bleach.
9. Perform Post Run UV Maintenance following the on-screen prompts.

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