



Intermountain Forensics

SOP #	EXT-201
Revision #	02

Forensic DNA Technical Leader Approval

Issue Date

Dana E Walker

03/01/2023

Differential Extraction - QIAcube EZ1 DNA Investigator

1. Purpose

This document describes the procedures for performing a differential separation of a mixture of sperm cells from non-sperm cells predominantly in sexual assault kit evidence processing.

2. Summary

Describes pre-processing reagent preparation. The materials and protocol for differential extractions are provided. This protocol utilizes the Qiagen EZ1 Advanced XL Extraction instrument for DNA extraction of both epithelial and sperm cells after differential separation by the Qiagen QIAcube Connect liquid handling robot.

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 dH₂O or TE to the vial of carrier RNA (310µg), provided in the EZ1 DNA Investigator kit.
 - b. Transfer entire volume into 20ul aliquots
 - c. Store the aliquots 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 QIAcube Diff tab of the saved Case Chronicle.
3. Enter the following information into the header of the document:
 - a. Batch ID
 - b. Performed by
 - c. Date of extraction



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d. Reagent Lot Numbers

4. Enter the sample identification numbers for all samples that will be extracted in the batch
5. Enter a reagent blank named with the following name convention: Numerical portion of the IMF Case Number without the place holder zeroes – Batch ID – RBD (Example: 231-230215SW-RB) between each set of case samples included on the tray.

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

Differential Digest

1. Warm MTL buffer at 70° C.
2. 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.
3. Opening each tube individually, add 500µL to each tube containing sample.
4. 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.
5. Incubate at 56°C for a minimum of 1 hour at 900rpm in a thermomixer.
 - a. The sample can be incubated up to 2 hours, based on the nature of the sample and any previous analysis results, if available.
6. If spin basket is used, centrifuge the tube at maximum centrifuge speed for 5 minutes. Discard the spin basket. If a spin basket is not used, briefly centrifuge to remove drops from the inside of the lid. Transfer all liquid to a new tube.
 - a. A Lyse and Spin Basket cannot be used with a differential sample.

QIAcube Differential Separation

1. Turn the instrument on by clicking the toggle switch on the back panel of the instrument, next to the power cable.
2. Using the touch screen, select **DNA** from the Applications options.
3. Select **Pipetting > Epithelial and Sperm Cell**
4. Select the **“3. Separation and Lysis”**.



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- a. "3. Separation and Lysis A" and "3. Separation and Lysis B" introduces a pause step that will allow removal of the Epithelial fraction tubes before the sperm pellet washes and may be selected instead of the method above.
- 5. Select **Next** to acknowledge the protocol cannot be modified.
- 6. Select the appropriate number of samples.
 - a. Note: A QIACube run cannot be performed if the batch total is 1 or 11 tubes.
- 7. Follow the instrument instructions for loading and placement of tubes, tips, and reagents.
 - a. Fill both tip racks with "Disposable Filter-Tips, 1000ul, **wide bore**".
 - b. Prepare Sperm lysis buffer in 2mL Tube Position A. See table below for minimum volumes.
 - a. The sperm lysis buffer may be prepared during "3. Separation and Lysis A" and loaded prior to starting "3. Separation and Lysis B".

# of Samples	Total Volume	G2	Pro-K	DTT
2	360	270	18	72
3	530	398	27	106
4	675	506	34	135
5	830	623	42	166
6	1000	750	50	200
7	1150	863	58	230
8	1300	975	65	260
9	1475	1106	74	295
10	1625	1219	81	325
12	1960	1470	98	392

- c. Aliquot Buffer G2 in 30mL reagent bottle in Reagent Bottle Rack position 1. See table below for minimum required volumes:

# of Samples	G2
2	6,620
3	8,680
4	10,740
5	12,800
6	14,860
7	16,920
8	18,980
9	21,040
10	23,100
12	27,220



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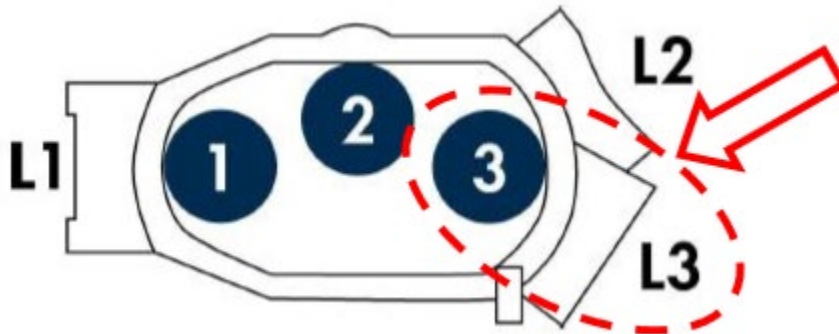
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- d. Place clean, empty 2mL tubes in Shaker, as directed by the instrument prompt for Epithelial Fraction collection.
8. Place lysed samples in position 3 of the QIAcube rotor adapter, with lid in position L3.



9. Place rotor adapters containing samples into QIAcube centrifuge as directed by the instrument prompt.
10. Close the instrument lid and select **Start**.
11. After the run, the Epithelial Fraction will be found in the 2mL tubes in the QIAcube shaker and the Sperm Fraction with 200µL lysis buffer will be found in the 1.5mL tubes in rotor adapter position 3.
 - a. If 3A and 3B are run separately, remove the Epithelial Fraction tubes at the conclusion of 3A and start the 3B protocol.
12. Prepare fractions for the EZ1 Instrument Purification
 - a. Epithelial Fraction:
 - i. Add 400µL of heated MTL Buffer to each Epithelial Fraction tube.
 - b. Sperm Fraction:
 - i. Incubate the Sperm Fraction tubes at 70°C for 10 min at 900 rpm in the thermomixer.
 - ii. Centrifuge the tube briefly to remove drops from inside the lid.

EZ1 Instrument Run for Fraction 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 worktable according to the messages shown in the display.
 - 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.



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- 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 the tubes, mark each tube 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.
5. Select the following parameters for the instrument run:
- a. Epithelial Fractions:
 - i. Press “3” for the “Large Volume Protocol”
 - ii. Press “1” to elute in water
 - iii. Press “1” to for a 40 µL elution volume
 - b. Sperm Fractions:
 - i. Press “1” for the “Trace Protocol”
 - ii. Press “1” to elute in water
 - iii. Press “1” to for a 40 µL elution volume
6. 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.
7. Close the EZ1 door.
8. Press **START** to start the protocol run.
9. After the run is finished, remove 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 due to the risk of the bleach and sodium thiocyanate creating cyanide gas fumes.
10. Perform Post Run UV Maintenance following the on-screen prompts.

4. References

05/2020 PROM-16287-001 Automated differential wash protocol using the QIAcube® Connect and the EZ1® DNA Investigator® Kit (User-Developed Protocol) EZ1® Advanced XL User Manual
EZ1® DNA Investigator® Handbook
QIAcube® Connect User Manual

5. Definitions

N/A



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