Differential Extraction - QIAcube EZ1 DNA Investigator

1. *Purpose*

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

2. *Summary*

Reagent preparation is described. Materials and protocol differential extractions are provided. The protocol utilizes the Qiagen EZ1 Advanced XL Extraction instrument for DNA extraction of both epithelial and sperm cells and the Qiagen QIAcube Connect liquid handling robot for differential separation.

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.

QIAcube Connect 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.

6. Incubate at 56°C for 1 to 2 hours at 900rpm in a thermomixer.

7. 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.

The QIAcube

8. Run QIAcube Separation and Lysis protocol, “3. Separation and Lysis”.
    a. Alternatively, running “3. Separation and Lysis A” and “3. Separation and Lysis B” will allow removal of the Epithelial fraction tubes before the sperm pellet washes.

9. Follow the instrument instructions for loading and placement.
    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

# 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

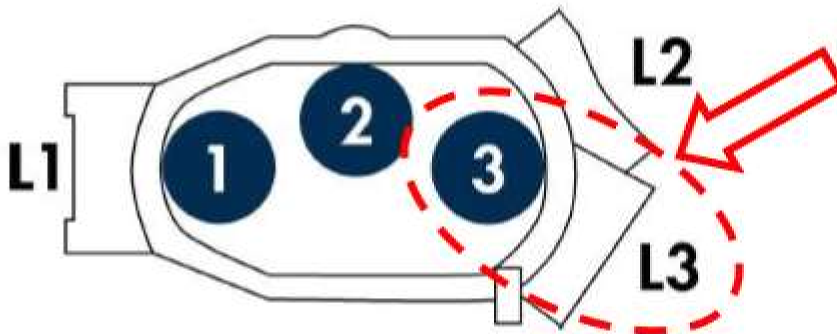
NOTE: The sperm lysis buffer may be prepared during "3. Separation and Lysis A" and loaded prior to starting "3. Separation and Lysis B".

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

d. Place clean, empty 2mL tubes in Shaker, as directed by the instrument prompt for Epithelial Fraction collection.

10. Place lysed samples in position 3 of the QIAcube rotor adapter, with lid in position L3.



11. Place rotor adapters containing samples into QIAcube centrifuge as directed by the instrument prompt.

12. Start run.

13. 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.

14. Add 400µL of heated MTL Buffer to each Epithelial Fraction tube. The Epithelial Fraction tubes are ready for the EZ1 instrument.

15. Incubate the Sperm Fraction tubes at 70°C for 10 min at 900 rpm in the thermomixer.

16. Centrifuge the tube briefly to remove drops from inside the lid. The Sperm Fraction tubes are ready for the EZ1 instrument.

EZ1

The Investigator XL Flip-Cap card must be used for the flip top tube rack.

Starting a run:

17. Open the EZ1 door, press **START** on the control panel, and 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.
    b. Load the reagent cartridges into the cartridge rack.

       Note: After sliding a reagent cartridge into the cartridge rack, ensure that you press down on the cartridge until it clicks into place.

    c. Load opened elution tubes into the first row of the tip rack.
    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.
    f. Large Volume Protocol is used for Epithelial Fractions.
       Trace Protocol is used for Sperm Fractions.

18. Close the EZ1 door. The protocol run cannot start until the door is closed.

19. Press **START** to start the protocol run.

20. After the run is finished, remove the elution tubes, which contain the purified samples, and discard the sample-preparation waste and used cartridges.

21. Perform Post Run Maintenance.

4. References

Automated differential wash protocols from QIAGEN
EZ1® Advanced XL User Manual
EZ1® DNA Investigator® Handbook
QIAcube® Connect User Manual

5. Definitions

N/A