User-Developed Protocol

Automated differential wash protocol using the QIAcube® Connect and the EZ1® DNA Investigator® Kit

This protocol has been adapted to allow the user to replace the epithelial transfer and sperm pellet wash steps with an automated protocol using the QIAcube Connect. With the addition of an extra tip rack position on the QIAcube Connect, the QIAcube protocols “Separation and Lysis 12 A” and “Separation and Lysis 12 B” can be combined into a single protocol when 3x sperm pellet washes are utilized. Additionally, the QIAcube Connect includes an updated graphical user interface (GUI) that facilitates the deck loading and run set-up. The epithelial and sperm fractions can then be purified on the EZ1 Advanced XL using the EZ1 DNA Investigator Kit

Note: This differential wash protocol uses Buffer G2 as a wash buffer. Additional Buffer G2, cat. no. 1014636, may be purchased separately.

This protocol has not been thoroughly tested and optimized by QIAGEN.

IMPORTANT: Please read the “Safety Information” and “Important Notes” sections in the *EZ1 DNA Investigator Handbook*, **www.qiagen.com/HB-0122**, before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate safety data sheets (SDSs), available from the product supplier. The EZ1 DNA Investigator Kit is intended for research use only. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

Equipment and reagents to be supplied by user

- QIAcube Connect (cat. no. 9002864)
- Filter-Tips, 1000 µl, wide-bore (cat. no. 990452)
- Rotor Adapters (10 x 24) (cat. no. 990394)
- 1.5 ml QIAcube Connect elution tubes (provided with QIAcube rotor adapters) or 1.5 ml Micro tube (Sarstedt cat. no. 72.690)
- 2 ml Sample Tubes CB (cat. no. 990382)
- EZ1 Advanced XL with appropriate DNA Investigator Protocol Card
- EZ1 DNA Investigator Kit (cat. no. 952034)
- Buffer MTL (cat. no. 19112) (may be warmed in aliquots in 2 ml tubes)
- Buffer G2 (cat. no. 1014636)
- Buffer ATL (cat. no. 19076)
- Dilution Buffer (10 mM Tris·Cl, 10 mM EDTA, 50 mM NaCl; alternatively, Tris-EDTA buffer, pH 8.0 (10 mM Tris, 0.1 M EDTA), or TE^{-4} may be used)
- 1 M dithiothreitol (DTT)
- Molecular-grade water, if applicable
- Pipette tips (pipette tips with aerosol barriers to prevent cross-contamination are recommended)
- 10–1000 µl pipettors
- Microcentrifuge
- Thermomixer or shaker incubator

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Important points before starting

- Refer to the *QIAcube Connect User Manual*, **www.qiagen.com/HB-2594**, for the correct loading of sample tubes in the rotor adapter and shaker.

Things to do before starting

- Prepare Dilute Buffer ATL (dATL) as follows: 1/3 volume Buffer ATL with 2/3 volume Dilution Buffer
- Heat a thermomixer or shaker-incubator to 56°C for the epithelial digest in Step 4 and to 70°C to warm Buffer MTL.

  Note: When QIAcube Connect is not in use, the onboard heater/shaker can be used independently. To use the heated shaker of the QIAcube Connect, press the **Tools** icon. Under the **Run Modules** tab, select **Heater Shaker**. Set the incubation settings by selecting the appropriate parameters on the screen.

Procedure

Sample preparation

1. Place the forensic sample in an appropriate microfuge tube.

   Note: A QIAcube Connect 1.5 ml elution tube may be used; however, this can lead to an increased risk of contamination when loading the elution tube into the QIAcube Connect rotor adapter.
2. Add 480 µl dATL to the sample.
3. Add 20 µl Proteinase K and mix thoroughly by vortexing for 10 s.

   Optional: Buffer dATL and Proteinase K may be prepared in a Master Mix, with 500 µl added per sample.
4. Incubate samples at 56°C for 1.5 to 2 h in a thermomixer, while shaking at 900 rpm.
5. Centrifuge the tube briefly to remove droplets from inside the lid.
6. Remove any solid material from the tube.

   Note: The sample volume should be approximately 500 µl.

   Note: If the substrate removed is to be processed, please proceed directly to step 15. Simply remove the solid material prior to loading the sample in the EZ1 Advanced XL.

Loading the QIAcube Connect

7. Select the appropriate separation and wash protocol and number of samples.
   a. After logging into the QIAcube Connect, select **DNA** from the “Applications” icon list.
   b. Select **Pipetting** > **Epithelial and Sperm Cell**.
   c. Select the appropriate protocol.
   d. Select **Next** to acknowledge the protocol cannot be modified
   e. Select the appropriate number of samples
8. Ensure that 2 ml tubes, Buffer G2 (and water, if applicable), and wide-bore tips are loaded into the QIAcube Connect as indicated by the selected protocol.

   Be sure to follow directions shown on the QIAcube Connect GUI for deck loading.

9. If the selected QIAcube Connect protocol will include the addition of SLB, then prepare a Master Mix of Buffer G2, Proteinase K, and 1M DTT (Table 1).

Table 1. Master Mix preparation for protocol with SLB

No. of samples	Buffer G2 (µl)	Proteinase K (µl)	1M DTT (µl)	Total volume (µl)
2	259.5	17.3	69.2	346
3	374.25	24.95	99.8	499
4	489	32.6	130.4	652
5	603.75	40.25	161	805
6	718.5	47.9	191.1	958
7	833.25	55.55	222.2	1111
8	948	63.2	252.8	1264
9	1062.75	70.85	283.4	1417
10	1177.5	78.5	314	1570
12	1407	93.8	375.2	1876

10. Transfer the lysate (with substrate removed) to a clean 1.5 ml QIAcube Connect elution tube that has been preloaded in position 3 and the lid in position L3 of the QIAcube Connect rotor adapter (Figure 1).

    Make sure to pipette-mix when aspirating the lysate to ensure an efficient transfer of the sperm pellet.

    Note: If the initial lysis in step 4 was conducted in a 1.5 ml QIAcube Connect elution tube, avoid contact with any of the internal surfaces of the tube or lid to reduce the risk of contamination.

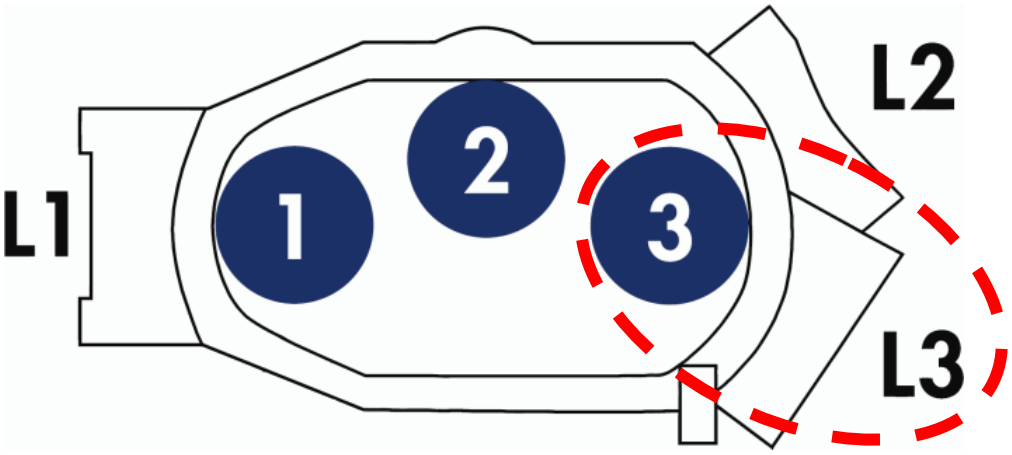

Figure 1. QIAcube Connect Rotor Adapter.

11. Place rotor adapters containing samples into the QIAcube Connect centrifuge.
12. Close the instrument lid and select **Start**.
13. At the end of the differential wash protocol, whether completed in a single protocol or split into 2 parts, the non-sperm fractions will be found in their respective positions in the shaker rack. The sperm fractions will be found in the 1.5 ml tubes in rotor adapter position 3

DNA purification

14. Non-sperm fraction:
    a. Add 400 µl prewarmed Buffer MTL to the 2 ml sample tube and gently pipette-mix.
    b. Place directly onto the appropriate row of the EZ1 sample rack.
    c. Run the EZ1 protocol “DNA Purification (Large-Volume Protocol)”.
15. Sperm fraction:

    Note: If the addition of SLB is not part of the QIAcube Connect program, manually add 145 µl of SLB to the sperm pellet. Prepare the SLB according to Table 1.

    a. Vortex vigorously for 10 s.
    b. Incubate samples at 70°C for 10 min in a thermomixer, while shaking at 900 rpm.
    c. Vortex vigorously for 10 s.
    d. Remove the snap-cap lid from the QIAcube 1.5 elution tube. Alternatively, the lysate may be transferred to a 2 ml EZ1 sample tube (included in the EZ1 DNA Investigator Kit).
    e. Run the EZ1 protocol “DNA Purification (Trace Protocol)”.

QIAGEN kit handbooks can be requested from QIAGEN Technical Services or your local QIAGEN distributor. Selected kit handbooks can be downloaded from **www.qiagen.com/resources**.

Safety data sheets (SDS) for any QIAGEN product can be downloaded from **www.qiagen.com/support/qa-qc-safety-data/safety-data-sheets**.