

**SCREENING FOR MALE DNA USING THE QIAGEN EZ1 DNA TISSUE KIT  
WITH THE BIOROBOT EZ1**

Date: 5/8/06

REV: 1

Approved: *ke*

**Purpose:**

This procedure may be used for extraction of DNA from sexual assault kits which may contain male DNA.

**Materials:**

1.5 mL snap cap tubes with deep well removable caps

Qiagen EZ1 DNA Tissue Kit containing: Reagent Cartridges, Disposable Tip Holders, Disposable Filter Tips, 2 mL Sample Tubes, 1.5 mL Elution Tubes, G2 Digest Buffer, and 20 mg/mL Proteinase K

1M Dithiothreitol (DTT)

Forceps, scissors, scalpel

Extraction QC sample

50X TE

Sterile nanopure water

**Equipment:**

Micropipettors and appropriate aerosol resistant tips

Microcentrifuge

Dry Heat block preheated to 56° C

Vortex

Qiagen BioRobot EZ1 with Forensic Card

Refrigerator

**Procedure:**

1. Label 1.5 mL tube with deep well cap twice. Place stained material in tube. When performing extractions, a reagent control (RC) and a quality control (QC) sample consisting of a semen stain must be included for each unknown sample set. [Recommended sample size: No more than ¼ of each swab. Aim for 1/8 of each swab. Smaller sample size is recommended to save most of sample for DNA analysis.]
2. Add the following:  
180 µL G2 Digest Buffer (provided in Qiagen EZ1 DNA Tissue Kit)  
10 µL of 20 mg/mL Proteinase K (provided in Qiagen EZ1 DNA Tissue Kit)  
10 µL of 1M Dithiothreitol (DTT)
3. Vortex briefly and incubate at 56° C for 1.5 to 24 hours.
4. Transfer the liquid digest (190-200 µL) from the E2 fraction to 2 mL sample tubes (provided in Tissue Kit).

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(The following steps deal with the set-up and operation of the BioRobot EZ1.)

5. Turn on the Qiagen BioRobot EZ1 with inserted Forensic Card.
6. Press 'Start' to display the protocols menu. Select the 'Trace Sample' protocol. Select TE elution buffer. Select 50  $\mu$ L elution volume.
7. Press any key to proceed through the text displayed in the LCD. (The text summarizes the following steps which describe the loading of the Biorobot EZ1.)
8. Open BioRobot EZ1 workstation door and remove worktable and cartridge rack.
9. Invert the reagent cartridges (1-6 per extraction run) to mix and tap the cartridges to deposit the reagents to the bottom of their wells. Load reagent cartridges into the cartridge rack.
10. Load the cartridge rack and worktable into the BioRobot EZ1.
11. Load (1-6) appropriately labeled opened elution tubes into the first row of the BioRobot EZ1 worktable.
12. Load (1-6) tip holders/filter tips into the second row of the BioRobot EZ1 worktable.
13. Load (1-6) appropriately labeled opened sample tubes into the fourth row of the worktable. Check to insure the sample tubes are in order corresponding to the elution tube order in the first row.
14. Close the workstation door and press 'Start' to begin the extraction protocol.
15. When the protocol ends, the LCD displays 'Protocol Finished'. Open the workstation door and remove the elution tubes containing the purified DNA. Proceed to Quantifiler Y Human Male DNA quantitation.
16. To run another protocol, press 'ESC' to return to the Protocols menu. Otherwise, press 'Stop' twice to return to the first screen of the LCD and perform the daily maintenance procedure outlined in AN DNA B18.

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**Results:**

No recordable results are obtained from this procedure. The material isolated using this procedure is used in subsequent procedures.

**Conclusions:**

Not applicable

**References:**

Qiagen BioRobot EZ1 DNA Handbook. Second Edition, February 2004.

Qiagen BioRobot EZ1 User Manual. January 2003.

Validation on file