

## QIAGEN Supplementary Protocol:

### Isolation of DNA from buccal cells using the EZ1 DNA Tissue Kit

This protocol is designed for the isolation of total (genomic and mitochondrial) DNA from buccal cells using the EZ1 DNA Tissue Kit in combination with the BioRobot® EZ1 workstation.

#### Introduction

The BioRobot EZ1 System allows fully automated purification of total DNA from buccal cells. The magnetic particle technology used by the BioRobot EZ1 System provides high-quality DNA, which is ideal for genotyping and epidemiological studies as well as forensic analyses. The isolated DNA is well suited for direct use in downstream applications, such as amplification or other enzymatic reactions. The BioRobot EZ1 performs all steps of the DNA isolation procedure.

This protocol describes first how to collect buccal cell samples (using either cotton swabs or brushes); this is followed by the procedure for harvesting and lysis of cells (using proteinase K) and the simple procedure for setting up the BioRobot EZ1 and starting a run.

**IMPORTANT:** Please read the *BioRobot EZ1 Genomic DNA Kit Handbook*, paying careful attention to the Safety Information and Important Notes sections, before beginning this procedure.

#### Starting material

Collection of buccal (or epithelial) cells from the inside of the cheek is a simple, inexpensive way to collect material for DNA isolation. Buccal cell samples may be processed on the same day as collection or stored for future processing. While storage at  $-20^{\circ}\text{C}$  is recommended, DNA of suitable quality for single-copy gene amplification has been documented from swabs stored at room temperature for 24 months (see Table 2). The starting and elution volumes to use in this procedure are given in Table 1, below.

**Table 1. Amount of starting material and elution volume for the EZ1 genomic DNA buccal cell procedure**

Sample type	Protocol	Starting volume	Elution volume
Buccal cells collected on swab or brush	Buccal Cells	200 $\mu\text{l}$ proteinase K-digested sample*	100 $\mu\text{l}$

\* See the procedure below.

#### Yield of purified DNA

Typical yields of DNA obtained from fresh and stored buccal cell samples are shown in Table 2, below.

**Table 2. DNA yields obtained from fresh and stored buccal cell samples using the BioRobot EZ1 System**

<b>Sample type</b>	<b>No. of samples tested</b>	<b>DNA yield (<math>\mu\text{g}</math>)</b>
Fresh buccal cell swabs	18	$2.78 \pm 0.92$
24-month-old buccal cell swabs*	6	$0.77 \pm 0.18$
24-month-old buccal cell brush samples*	15	$1.47 \pm 0.40$

\* Samples were stored (air-dried) at room temperature for 24 months.

### **Equipment and reagents required**

- BioRobot EZ1
- EZ1 DNA Tissue Kit (48), cat. no. 953034
- EZ1 DNA Buccal Swab Card, cat. no. 9015589

### **Important notes before starting**

- This protocol has been tested using the following swab types: plastic swabs with cotton or Dacron<sup>®</sup> tips. (Puritan<sup>®</sup> applicators with plastic shafts and cotton or Dacron tips are available from: Hardwood Products Company, [www.hwppuritan.com](http://www.hwppuritan.com), item nos. 25-806 1PC and 25-806 1PD; and from Daigger, [www.daigger.com](http://www.daigger.com), cat. nos. EF22008D and EF22008DA). Nylon cytology brushes and other swab types may also be used.
- Before use, dilute Buffer G2 in distilled water using a ratio of 1:0.5 (i.e., one volume of Buffer G2 to 0.5 volumes of distilled water) for n+1 samples (where n is the number of samples to be digested). Buffer G2 may also be used undiluted although, due to the increased volume of Buffer G2 required for the buccal cells protocol, fewer isolations will be possible. Use of diluted Buffer G2 does not influence DNA yield or quality.

### **Sample collection**

To collect a sample, scrape the swab or brush against the inside of each cheek 6 times. Allow the swab or brush to air-dry for at least 2 h after collection. Ensure that the person providing the sample has not consumed any food or drink for 30 min prior to sample collection.

### **Procedure**

This procedure involves harvesting of cells from the buccal swab or brush and lysis using QIAGEN Proteinase K, and subsequent automated isolation of DNA using the BioRobot EZ1.

## Proteinase K digestion of buccal cells

1. **Carefully cut or break off the end part of the swab or brush into a 2 ml sample tube (with screw cap), using an appropriate tool (e.g., scissors). Add 290  $\mu$ l of diluted Buffer G2 to the sample.**

**Note:** Prepare diluted Buffer G2 as described above in "Important notes before starting".

2. **Add 10  $\mu$ l Proteinase K, and mix thoroughly by vortexing for 10 s.**

If processing buccal cell brush samples, centrifuge the tube briefly (at 10,000 x g for 30 s) to force the brush to the bottom of the tube.

3. **Incubate at 56°C for 15 min.**

Vortex the tube 1–2 times during the incubation, or place in a thermomixer.

4. **Centrifuge the tube briefly to remove drops from inside the lid.**

5. **Remove the swab or brush from the tube.**

Using forceps, press the swab or brush against the inside of the tube to obtain maximum sample volume. The sample volume should be approximately 200  $\mu$ l.

## DNA isolation

6. **Insert the EZ1 DNA Buccal Swab Card completely into the EZ1 Card slot of the BioRobot EZ1.**

7. **Switch on the BioRobot EZ1.**

8. **Press "START" to display the "Protocols" menu.**

9. **Press "1" to start worktable setup.**

10. **Press any key to proceed through the text displayed in the LCD.**

The text summarizes the following steps which describe the loading of the worktable.

11. **Open the workstation door.**

12. **Invert 1–6 reagent cartridges twice to mix the magnetic particles. Then tap the cartridges to deposit the reagents at the bottom of their wells.**

13. **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.

If there are fewer than 6 reagent cartridges, you can load them in any order on the rack. However, when loading the other labware in steps 14–16, ensure that they also follow the same order.

14. **Load 1–6 opened elution tubes into the elution tube rack.**

15. **Load 1–6 tip holders containing filter-tips into the front row of the tip rack.**

16. **Load 1–6 opened sample tubes containing buccal cell samples into the back row of the tip rack.**

17. **Close the workstation door.**

18. **Press "START" to start the purification procedure.**

The automated purification procedure takes 15–20 min.

**19. When the protocol ends, the LCD displays "Finished". If you intend to run another protocol, press "ESC" to return to the "Protocols" menu. Otherwise, press "STOP" twice to return the motors to their home positions.**

**20. Open the workstation door.**

**21. Retrieve the elution tubes containing the purified DNA. The DNA is ready to use, or can be stored at 2–8°C for 24 h or at –20°C for longer periods.**

If the purified DNA is to be analyzed by real-time PCR, tubes containing eluate should first be applied to a suitable magnetic separator and the eluate transferred to a clean tube (see the *BioRobot EZ1 Genomic DNA Kit Handbook* appendix) in order to minimize the risk of magnetic-particle carryover.

**22. Clean the BioRobot EZ1.**

Follow the maintenance instructions in the *BioRobot EZ1 User Manual*.

**23. If you pressed "ESC" in step 19, follow the procedure from step 9 onward to run another protocol. Otherwise, close the workstation door and switch off the BioRobot EZ1.**

## Troubleshooting

For general troubleshooting, please consult the Troubleshooting Guide in the *BioRobot EZ1 Genomic DNA Kit Handbook*. The troubleshooting described here is specific for the buccal cells application.

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### Comments and suggestions

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#### Low DNA yield

Insufficient number of cells in sample

When collecting buccal cell samples, be sure to scrape the swab or brush firmly against the inside of each cheek 6 times

EZ1 Kits are intended as general-purpose devices. No claim or representation is intended for their use to identify any specific organism or for a specific clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of EZ1 Kits for any particular use, since the performance characteristics of these kits have not been validated for any specific organism. EZ1 Kits may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

The BioRobot EZ1 is intended as a microtiter diluting and dispensing device. No claim or representation is intended for its use in identifying any specific organism or for a specific clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the user's responsibility to validate the performance of the BioRobot EZ1 for any particular use, since its performance characteristics have not been validated for any specific organism. The BioRobot EZ1 may be used in clinical diagnostic laboratory systems after the laboratory has validated their complete system as required by CLIA '88 regulations in the U.S. or equivalents in other countries.

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