

Preparing Buccal Swab Samples for DNA Purification

Materials to Be Supplied by the User

- benchtop vortex mixer
- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- 1.5–2.0ml tubes for incubation of samples (e.g., ClickFit Microtube, 1.5ml [Cat.# V1231])
- heating block set at 56°C
- **optional:** Clearing Columns (Cat.# Z3871)

Note: This kit has been tested with dry buccal swab samples stored at 15–30°C prior to DNA purification.

The total yield of genomic DNA from buccal swab samples depends on how well buccal cells are transferred to the swab and how many swabs are processed.

1. Prepare incubation tubes that will fit into the heating block.
2. **Optional:** Place a clearing column (Cat.# Z3871) in each incubation tube.
3. Place 1–2 buccal swab head(s) in each incubation tube or clearing column in each incubation tube. Remove the stick from the buccal swab head(s) by cutting or breaking the swab stick off above the swab head so that the cap can be closed on the tube or clearing column containing the swab head(s).
4. In a separate tube, combine 300µl of Lytic Enhancer (LE2) with 30µl of Proteinase K (PK) Solution for each sample plus one extra sample. See the table below.

Reagent	Amount per Reaction	Reactions (Sample Number + 1)	Total
Lytic Enhancer (LE2)	300µl	n + 1	300 × (n + 1)µl
Proteinase K (PK) Solution	30µl	n + 1	30 × (n + 1)µl

5. Mix the Lytic Enhancer (LE2)/Proteinase K (PK) Solution by inverting the tube at least 10 times.
6. Add 330µl of Lytic Enhancer (LE2)/Proteinase K (PK) Solution to each sample, and close the tube.
7. Incubate each tube in the 56°C heating block for 20 minutes. During this incubation, prepare cartridges as described below.
8. Use one of the following options to remove the swab head(s) from the tube:
 - a. If using a Clearing Column, place the tube in a microcentrifuge and centrifuge at maximum speed for 2 minutes. Remove the tube from the microcentrifuge. Open the tube; remove and discard the clearing column containing the swab head(s).
 - b. If not using a Clearing Column, use tweezers to remove the swab head(s) from the tube, carefully squeezing the remaining lysate from the swab head(s). Discard the swab head(s). Clean the tweezers and change gloves between each swab head removal to prevent cross contamination.
9. Add 300µl of Lysis Buffer to well #1 of each cartridge to be used (well #1 is the largest well in the cartridge).
10. Transfer each swab lysate sample from the incubation tube to well #1 of a separate cartridge and mix well with the Lysis Buffer and binding solution in well #1 by aspirating and dispensing 5–10 times after transfer to make a homogeneous mixture.

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Cartridge Preparation

1. Place the cartridge to be used in the deck tray with well #1 (the largest well in the cartridge) facing away from the elution position, which is the numbered side of the tray.
2. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing the cartridge in the instrument.
3. Add 15µl of RNase A Solution into well #3 of each cartridge.
4. Place a plunger in well #8 of each cartridge. Well #8 is the well closest to the elution tube.
5. Place an empty elution tube into the elution tube position for each cartridge. Add 50–200µl of Elution Buffer to the bottom of each elution tube.
Note: Use only the Elution Tubes (0.5ml) provided in the kit; other tubes may be incompatible with supported Maxwell[®] Instruments.
6. Follow the instrument run instructions in the *Maxwell[®] RSC Genomic DNA Kit Technical Manual #TM708*.



Figure 1. Setup and configuration of deck trays. Elution Buffer is added to the elution tubes as shown. Plungers are in well #8 of the cartridge. Deck tray shown is from the Maxwell[®] RSC Instrument (Cat.# AS4500).

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Additional protocol information is in Technical Manual #TM708, available online at: www.promega.com