User-Developed Protocol:

Isolation of total RNA from ejectable buccal swabs using the RNeasy® Micro Kit

This protocol has been adapted by customers for the isolation of total RNA from ejectable buccal swabs using the RNeasy Micro Kit. **It has not been thoroughly tested and optimized by QIAGEN.**

IMPORTANT: Please be sure to read the *RNeasy Micro Kit Handbook* before beginning this procedure, paying careful attention to the Safety Information section.

Reagents and equipment to be supplied by the user

- QIAshredder™ Homogenizer (Cat. No. 79654)
- C.E.P. swabs (e.g., Omni Swabs from Whatman Bioscience, www.whatman.com)
- 14.3 M β-mercaptoethanol (β-ME)* (commercially available solutions are usually 14.3 M)
- Ethanol (70 and 80%)
- Microcentrifuge (with rotor for 2 ml tubes)
- Sterile, RNase-free pipet tips

Important points before starting:

- All centrifugation steps are carried out at room temperature (15–25℃).
- To collect a sample, scrape a fresh swab firmly against the inside of each cheek 6 times. Ensure that the person providing the sample has not consumed any food or drink in the 30 min prior to sample collection. Place the fresh swab in to Buffer RLT within 2 hours after collection.
- Yield will depend on the quality of the buccal cells collected.

Things to do before starting

- β-ME must be added to Buffer RLT before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for 1 month after addition of β-ME.
- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96-100%), as indicated on the bottle, to obtain a working solution.
- Prepare DNase I stock solution by dissolving the solid DNase I (1500 Kunitz units) in 550 µl of the RNase-free water provided. Take care that no DNase I is lost when opening the vial. Mix gently by inversion. Do not vortex.
- Prepare a DNase I incubation mix (10 µl DNase I stock solution and 70 µl Buffer RDD)

Procedure

1. **Eject the buccal swab into a 1.5 ml microcentrifuge tube. Add 400 µl Buffer RLT and vortex for 1 min.**

   The ejectable swab is ejected into the microcentrifuge tube by pressing the stem end of the swab.

   Note: Ensure β-ME has been added to Buffer RLT (see "Things to do before starting").

2. **Transfer the lysate and swab to a QIAshredder Mini Spin Column (in a 2 ml collection tube). Centrifuge for 5 min at maximum speed.**

   Note: It is important to transfer the swab and the lysate on to the QIAshredder spin column, to avoid any loss of RNA.

3. **Add 1 volume (approx. 350 µl) of 70% ethanol to the homogenized lysate and mix well by pipetting. Do not centrifuge.**

4. **Apply the sample to an RNeasy MinElute™ Spin Column in a 2 ml collection tube (supplied). Centrifuge at ≥8000 x *g* for 15 s and discard the flow-through.**

5. **Add 350 µl Buffer RW1 to the RNeasy MinElute Spin Column and centrifuge at ≥8000 x *g* for 15 s. Discard the flow-through.***

6. **Pipet 80 µl of DNase I incubation mix directly onto the RNeasy MinElute silica-gel membrane. Incubate at room temperature (15–25°C) fo r 15 min.**

7. **Add 350 µl Buffer RW1 to the RNeasy MinElute Spin Column, and centrifuge at ≥8000 x *g* for 15 s. Discard the flow-through and collection tube.***

8. **Transfer the RNeasy MinElute Spin Column into a fresh 2 ml collection tube (supplied). Pipet 500 µl Buffer RPE onto the RNeasy MinElute Spin Column. Centrifuge at ≥8000 x *g* for 15 s and discard the flow-through.**

   Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting").

9. **Add 500 µl of 80% ethanol to the RNeasy MinElute Spin column. Centrifuge at ≥8000 x *g* for 2 min. Discard the flow-through and collection tube.**

   Prepare the 80% ethanol with ethanol (96–100%) and the RNase-free water supplied with the kit.

10. **Transfer the RNeasy MinElute Spin Column to a new 2 ml collection tube (supplied). Open the cap of the spin column, and centrifuge in a microcentrifuge at full speed for 5 min.**

11. **To elute the RNA, transfer the spin column to a new 1.5 ml collection tube (supplied). Pipet 14 µl RNase-free water directly onto the center of the RNeasy silica-gel membrane. Centrifuge at maximum speed for 1 min.**

* Flow-through contains buffer RW1 and is therefore not compatible with bleach. See the RNeasy Micro Handbook for safety information.

QIAGEN® kit handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor.
Selected kit handbooks can be downloaded from www.qiagen.com/literature/
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp