Promega

Product Application

Automated purification of DNA from ESwab™ Samples

Purify DNA from Eswab™ swab and 1ml of transport media using the Maxwell® RSC instrument and Maxwell® RSC Stabilized Saliva DNA Kit

Kit: Maxwell® RSC Stabilized Saliva DNA Kit (Cat.# AS1630)

Analyses: qPCR to eubacterial 16S target

Sample Type(s): ESwab™ used to swab cell phones

Materials Required:

- Maxwell® RSC Stabilized Saliva DNA Kit (Cat.# AS1630)
- Maxwell® RSC instrument (Cat.# AS4500)
- Heat block at 56°C
- ESwab™ Swabs and Collection Devices (Copan USA Cat.# 480C)
- 2.0ml Eppendorf Tubes (Eppendorf Cat.# 022363352)
- Lysis Buffer (Part.# MC501)
- Proteinase K (Part.# MC500)
- Clearing Columns (Part.# Z387A)
- Click-Fit Tubes (cat.# V4741)

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

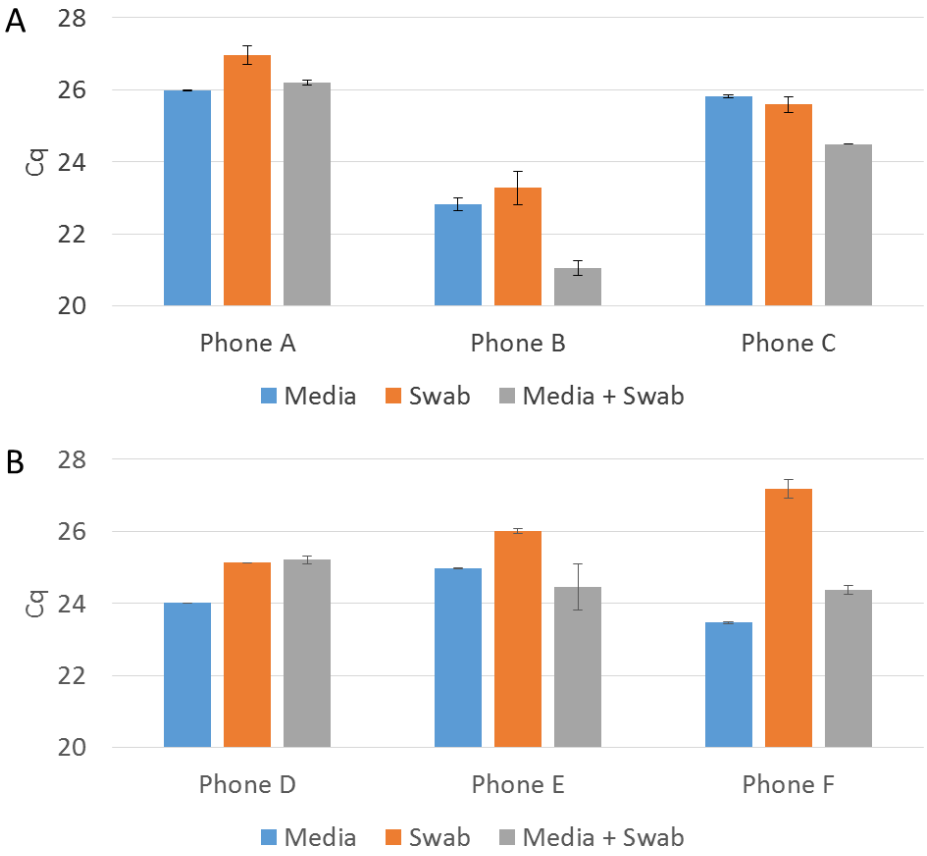
For further information, see Technical Manual TM480, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Protocol:

1. Use 2ml processing tubes for lysis. To each ESwab™ sample (~ 1ml total volume), add 300µl Lysis Buffer and 30µl of Proteinase K (Pro K). Vortex samples for 10 seconds to mix. Incubate samples for 20 minutes at 56°C.
2. After lysis, transfer swabs to a Clearing Column in a Click-Fit Tube and centrifuge in a microcentrifuge at maximum speed for two minutes to recover the lysate volume from the swab. Combine the recovered lysate with the remaining lysate in the 2ml processing tubes.
3. Transfer the entire volume of lysate to well #1 of a Maxwell® RSC Stabilized Saliva DNA Kit Cartridge. Add 50µl of Elution Buffer to the elution tube. Process samples using the Buccal Swab DNA Method on the Maxwell® RSC instrument.
4. If samples are cloudy, centrifuge them for 3 minutes at maximum speed, and transfer the cleared eluate to a separate tube.

Results:



DNA yields from phone swabs. Phones were swabbed with two swabs, and Transport Media alone, Swab alone, or both Media and Swab were processed using the Maxwell® Stabilized Saliva DNA Kit with Lysis Buffer and ProK for upstream preprocessing as described in the methods. Each set of swabs was processed as three samples: (a) Media only – swab was squeezed on size of tube to release as much media as possible. Media was transferred to 2ml tube and lysed. (b) Swab only – swab from (a) was transferred to a separate 2ml tube and lysed. (c) Media + Swab – media and swab were transferred to a 2ml tube and lysed. The yields were determined in a qPCR assay using GoTaq® qPCR Master Mix (Cat.# A6001)with eubacterial primers to the 16S gene, and Cq values are shown. Data represents a single replicate for each sample. Panels A and B represent separate purifications by the same method and a separate set of amplification reactions, so they have not been combined into one figure. Error bars represent the standard deviation of duplicate amplification reactions.