Promega

Product Application

Automated purification of total nucleic acid (TNA) from dermatological tapes.

Total nucleic acid is purified from D-Squame dermatological tapes using the Maxwell® RSC Blood DNA Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC Blood DNA Kit (Cat.# AS1400)

Analyses: RT-qPCR and qPCR

Sample Type: D-Squame Sampling Discs

Input: up to 10 sampling discs

Materials Required:

- Maxwell® RSC Blood DNA Kit (Cat.# AS1400)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Nuclease-Free Water (Cat.# P1193)
- Heat block set to 56°C

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 TM419, available at: **www.promega.com/protocols**

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

Protocol:

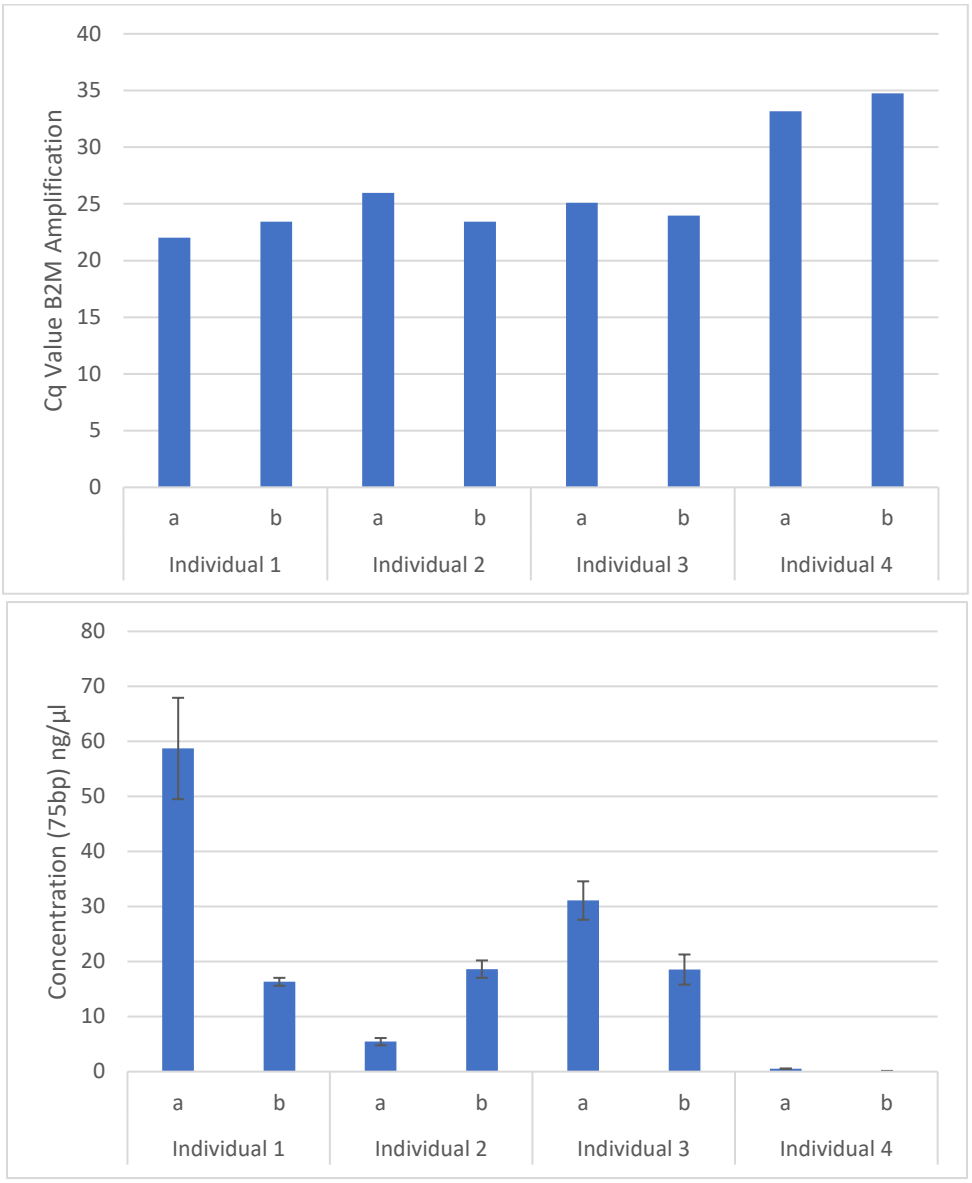
Note: This protocol assumes that the dermatological tapes have been collected in 1.5ml tubes (1 tape per tube) with the adhesive facing inward.

1. Prepare a lysis cocktail consisting of 300µl Lysis Buffer and 300µl Nuclease-Free Water; prepare sufficient volume of the lysis cocktail for X + 1 samples.

   Note: A single sample may consist of 1-10 dermatological tapes collected in individual tubes.
2. Add 600µl of the lysis cocktail to the first tube containing a tape.
3. Vortex the tube at maximum speed for 10 seconds.
4. Briefly centrifuge the sample to collect the liquid in the bottom of the tube. If processing multiple tapes, use a pipette to transfer liquid from the first tube into the next tube containing a tape. Do not discard the processed tube. If processing a single tape, continue to step 6 of this protocol.
5. Repeat starting at step 3 and continue until up to 10 tapes have been processed in this manner.
6. Spin all processed tubes for 1 minute at maximum speed in a microcentrifuge to collect the residual liquid in the bottom of the tubes. Combine the lysate from all processed tubes in a clean 1.5ml tube.
7. Add 30µl of Proteinase K to the lysate and vortex briefly to mix. Incubate at 56°C for 20 minutes. During the incubation step, prepare the Maxwell® cartridge according to the Technical Manual Maxwell® RSC Blood DNA Kit (TM419).
8. Add the entire volume of lysate to well #1 of the prepared Maxwell® RSC Blood DNA cartridge. Process samples on the Maxwell® RSC Instrument with the Blood DNA method.

Results:

Total nucleic acid (RNA and DNA) was recovered from 10 skin tapes using the protocol described above with the Maxwell® RSC Blood DNA Kit on the Maxwell® RSC Instrument. The figure below shows real time PCR detection of RNA and DNA purified from 10 tapes collected from 4 individuals in duplicate.



Real-time PCR detection of RNA and DNA purified from dermatological tapes collected from four individuals. Total nucleic acid was purified from 10 dermatological tapes as described above. **Top Panel.** RNA detection in TNA eluates from 10 tapes from four individuals. RNA was detected using the GoTaq® 1-step RT-qPCR System (Cat.# A6020) and primers to human ß-2-microglobulin (B2M). The average Cq value for duplicate amplification reactions is reported. **Bottom Panel.** DNA detection in TNA eluates from 10 tapes from four individuals. DNA was detected using the ProNex® DNA QC Assay ABI 7500/7500FAST (Cat.# NG1002), and results for DNA concentration based on the standard curve and amplification of the 75 base pair target are reported, average ± standard deviation (triplicate amplifications).