Promega

Product Application

DNA isolation from urine using the Maxwell® RSC Instrument

Isolate high quality, amplifiable DNA from urine using the Maxwell® RSC Blood DNA Kit on the Maxwell® RSC Instrument.

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

Analyses: UV absorbance, dye-based quantitation, qPCR, gel electrophoresis

Sample Type(s): Human urine

Materials Required:

- Maxwell® RSC Instrument (Cat.# AS4500)
- Maxwell® RSC Blood DNA Kit (Cat.# AS1400)
- 1X PBS
- Centrifuge (50ml conical tube capacity)

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**

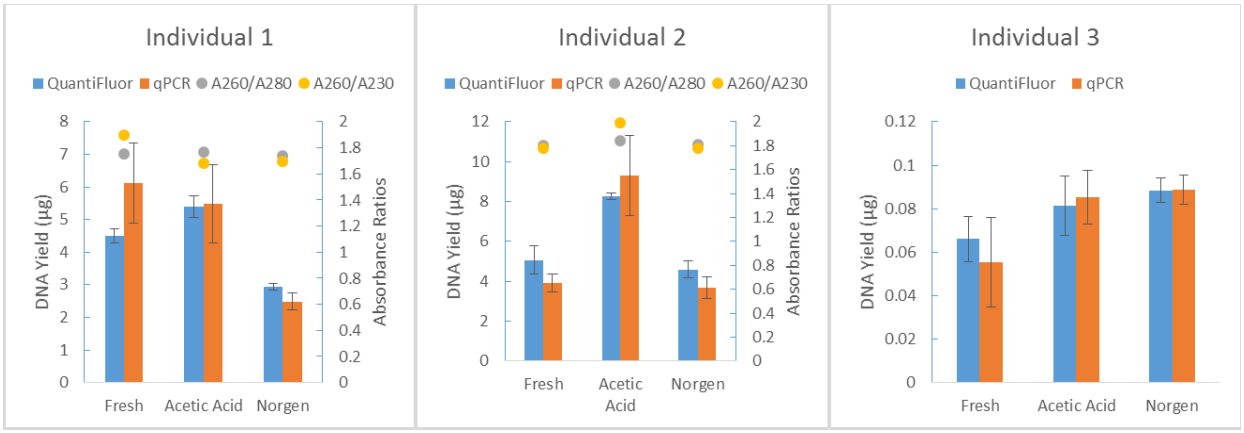
Protocol:

1. Collect urine in a sterile container. Either process urine immediately or add preservative for long term storage. Store urine at 4°C for short term storage.
2. Aliquot urine into 50ml conical tubes (Maximum of 50ml).
3. Spin samples in a centrifuge at 2000 *x g* for 20 minutes to collect cell pellet.
4. Remove supernatant and suspend pellet in 750µl of 1X PBS. Transfer cell suspension into a 1.5ml microcentrifuge tube.
5. Centrifuge tube at 10,000 *x g* for 2 minutes to collect cell pellet.
6. Remove PBS and suspend pellet in 300µl Lysis Buffer and 30µl Proteinase K Solution.
7. Vortex sample to fully suspend pellet.
8. Incubate at 56°C for 20 minutes.
9. Add entire lysate to well #1 of the Maxwell® RSC Blood Cartridge.
10. Place the cartridge in the Deck Tray and add plunger into well #8.
11. Add 50µl of Elution Buffer to the bottom of each elution tube and place on the Deck Tray.
12. Run the Maxwell® RSC Blood DNA method on the Maxwell® RSC Instrument.

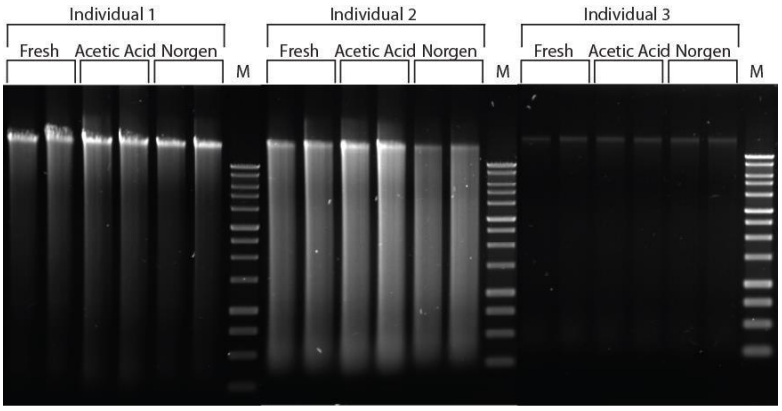
Results:

The above protocol was tested with 30ml of urine collected from 3 individuals. Each individual's urine was either processed immediately ("Fresh") or stored overnight in preservative. Preservation methods included 0.5% acetic acid (final concentration) and Norgen Biotek Urine Collection and Preservation Tubes. After isolating DNA using the Maxwell® RSC Blood DNA kit, DNA eluates were assayed for yield (QuantiFluor® ONE dsDNA system and qPCR), purity (A260/A280 and A260/A230 Absorbance ratios), amplifiability (qPCR), PCR inhibition, and quality/size (agarose gel electrophoresis).

A.



B.



1.0% agarose gel stained with ethidium bromide. M=Benchtop 1kb ladder.
10ul of each eluated was loaded (n=2)

C.

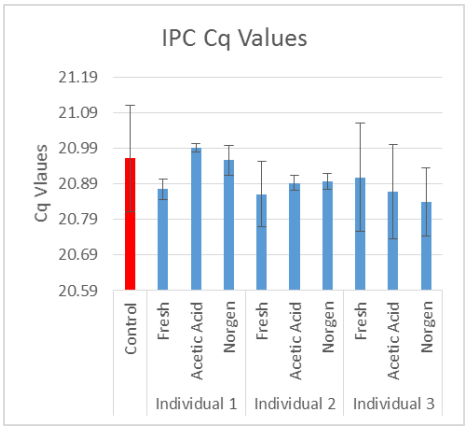


Figure 1. Isolation of DNA from 30ml of urine using the Maxwell® RSC Blood DNA Kit (Cat.# AS1400) on the Maxwell® RSC Instrument (Cat.# AS4500). A. DNA yield determined by QuantiFluor® ONE dsDNA System (Cat.# E4871) and by qPCR, and absorbance ratios (A260/A280 and A260/A230) (n=3). The absorbance ratios for individual 3 were below instrument limits and therefore not displayed. **B.** 1% Agarose gel using 10µl DNA eluates (n=2). **C.** PCR inhibition. Cq values for an IPC (Internal Positive Control) with DNA from purifications show no significant shift compared to control. Note: Individual 2 had very yellow urine and was collected over 12 hours. Individual 3 had very light colored urine and collected over ~4hrs. Individual 1 had urine color between Individual 1 and 3 and was collected over 12 hours. Individual 1 was male and 2/3 were female. Pellet sizes formed after centrifugation of 30ml of urine were 2>1>3.