

DNA Extraction from Whole Blood Spotted on Sample Collection Cards using the Maxwell® RSC DNA FFPE Kit

Purify gDNA from whole blood spotted on sample collection cards using the Maxwell® RSC DNA FFPE Kit coupled with Incubation Buffer

Kit: Maxwell® RSC DNA FFPE Kit (Cat.# AS1450)

Analyses: Dye-based quantitation, qPCR

Sample Type(s): 100µl of human blood spotted on cards

Input: One to three 3mm punches from cards

Materials Required:

- Maxwell® RSC DNA FFPE Kit (Cat.# AS1450)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Incubation Buffer (Cat.# D920F)
- DNA IQ™ Spin Baskets (Cat.# V1221)
- Heat block
- 1.5ml tubes
- Microcentrifuge

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 TM437, available at:
www.promega.com/protocols

or contact Technical Services at:
techserv@promega.com

Protocol:

1. Place 3mm punches (1 to 3 in total) into a 1.5ml tube.
2. Add 300µl of Incubation Buffer.
3. Add 25µl of Proteinase K and mix by pipetting.
4. Incubate at 56°C for 30 minutes.
5. Add entire contents of tube to a DNA IQ™ Spin Basket placed in a clean 1.5ml tube.
6. Spin the sample at maximum speed for 2 minutes.
7. Transfer the flowthrough to well #1 of the Maxwell® RSC Reagent Cartridge.
8. Place one of the supplied elution tubes into the sample rack and add 50µl of the supplied Nuclease-Free Water for each sample.
9. Place a plunger in well #8 of each cartridge.
10. Select method DNA FFPE in the Maxwell® RSC Instrument and Run.

Results: One hundred microliters of whole blood were spotted onto three different types of collection cards: FTA Classic Card Whatman, 930 Protein Saver Card Whatman and PerkinElmer 225. DNA was purified from one, two and three 3mm punches using the protocol above.

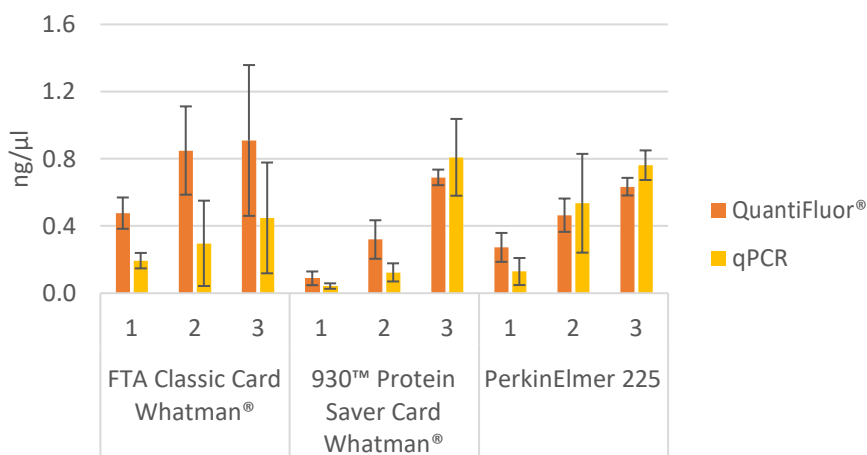


Figure 1. Average concentration of DNA purified from whole blood on multiple 3mm punches from different cards using the Maxwell® RSC DNA FFPE Kit. Concentration measured by QuantiFluor® ONE dsDNA Kit (Cat.# E4870) and qPCR based on amplification of the autosomal target with PowerQuant® System (Cat.# PQ5002). 1, 2 and 3 refers to the number of punches tested. N=3. Standard deviations are shown.

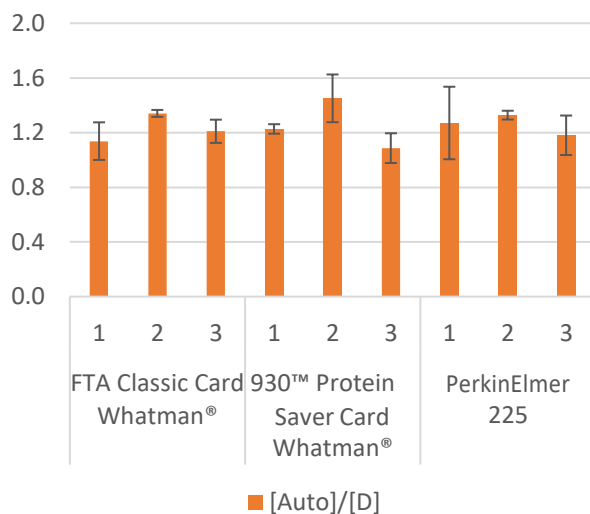


Figure 2. qPCR analysis of DNA purified from whole blood on multiple 3mm punches using the Maxwell® RSC DNA FFPE Kit and amplified using the PowerQuant™ System (Cat.# PQ5002) in a 20μl final volume. An [Auto]/[D] ratio close to 1 would suggest that no DNA degradation is evident.