Product Application

Automated Purification of ccfDNA from Plasma Isolated from Blood Collected in Streck Cell-Free DNA BCTs®

Purify ccfDNA from plasma isolated from blood collected in Streck Cell-Free DNA BCTs® using the Maxwell® RSC ccfDNA Plasma Kit, adding Proteinase K to the cartridge for increased yield.

Kit: Maxwell® RSC ccfDNA Plasma Kit (Cat.# AS1480)

Analyses: Electrophoresis, qPCR, Digital Droplet PCR

Sample Type(s): Plasma isolated from blood collected in Streck Cell-Free DNA BCTs®

Input: 1ml

Materials Required:

- Maxwell® RSC ccfDNA Plasma Kit (Cat.# AS1480)
- Streck Cell-Free DNA BCTs® (Streck Cat.# 218962)
- Proteinase K Solution, 20mg/ml (Cat.# MC5005)
- Sterile 15ml conical tubes

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 TM454, available at: **www.promega.com/protocols.**

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

Protocol:

1. Collect blood and immediately invert tubes gently 10 times to thoroughly mix preservation liquid. Inadequate mixing significantly increases the risk of white blood cell lysis.
2. When ready to process, invert tubes on a rotisserie device for 15 minutes and fractionate blood by centrifugation at 1,600 x *g* for 10 minutes.
3. Aspirate the top layer of plasma (avoiding the buffy coat layer) and place into a fresh 15ml conical tube.
4. Centrifuge the collected plasma at 2,000 x *g* for 20 minutes.
5. Again, aspirate the plasma and add to a fresh 15ml conical tube. Avoid disturbing the cell pellet.
6. Prepare Maxwell® RSC ccfDNA Plasma Cartridges as outlined in TM454.
7. Invert isolated plasma at least 10 times to mix.
8. Add 100µl of Proteinase K solution to well #1 of each cartridge followed by 1ml of plasma. Tip mix the plasma sample in well #1 to ensure all plasma is transferred.
9. Initiate run method as outlined in TM454.

Results:

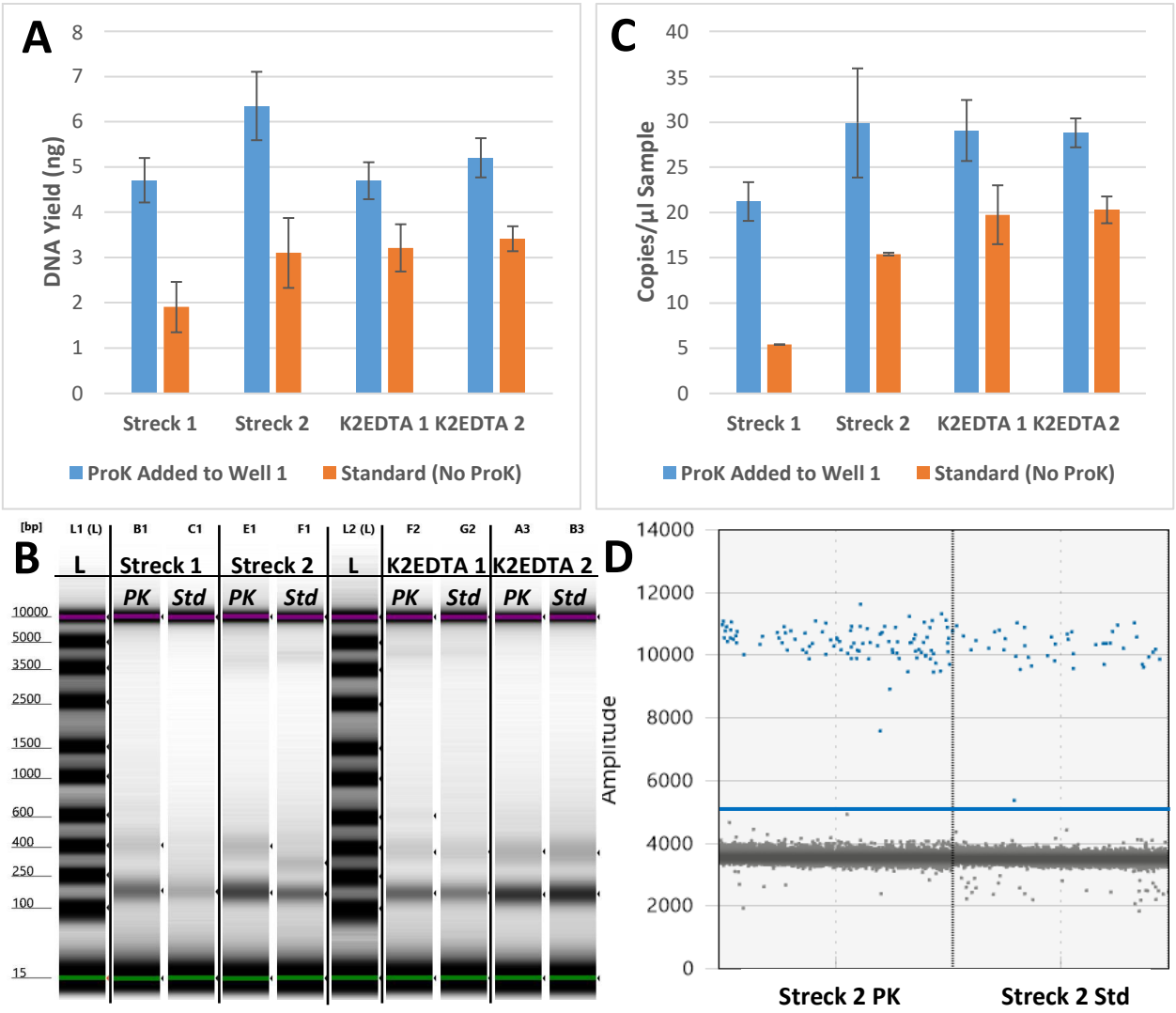

Figure 1. Yields, TapeStation results, and droplet digital PCR results for DNA purified from plasma isolated from Streck and K2EDTA tubes. Plasma was isolated from blood collected in Streck and standard K2EDTA tubes from two individuals (1 and 2). ccfDNA was then purified using the Maxwell® RSC ccfDNA Plasma Kit with two different methods: 1) Addition of Proteinase K (ProK) to well 1 of the Maxwell® cartridge (PK) and 2) standard protocol without ProK (Std). Mean DNA yields ± error based on the 75bp amplicon of the ProNex® DNA QC Assay (Cat.# NG1003) is shown in **Panel A** (n=3, except for Streck 1 Standard (n=2)). One replicate of each sample was run using the High Sensitivity D5000 ScreenTape Assay. The resulting gel image is shown in **Panel B** (L = ladder). Droplet digital PCR quantitation was performed following the Bio-Rad ddPCR™ Copy Number Variation Assay, Validated protocol, amplifying human genomic DNA target EIF2C1 (FAM). Mean copies of EIF2C1 target amplified per µl of purified ccfDNA input ± standard deviation is shown in **Panel C** (n=3 for ProK samples, n=2 for Standard samples except for Streck 1 Standard (n=1)). **Panel D** displays an example 1D droplet plot for the EIF2C1 target. The threshold, above which droplets are considered positives (contain target DNA), is shown as a blue line. The Y-axis is relative fluorescence. Each column contains the droplet plot for the sample on the x-axis.