Promega

Product Application

ccfDNA Purification from Plasma Samples

Isolate high quality, amplifiable ccfDNA from plasma samples using a manual purification system.

Kit: ReliaPrep™ FFPE gDNA Miniprep System (Cat.# A2351)

Analyses: ProNex® DNA QC Assay BioRad CFX96™ (Cat.# NG1004)

Sample Type(s): Human Plasma

Input: 300µL-1mL of Plasma

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.

or by e-mailing Technical Services techserv@promega.com

Materials Required:

- ReliaPrep™ FFPE gDNA Miniprep System (Cat.# A2351)
- Proteinase K (Cat.# MC5005)
- BL Buffer (Cat.# Z103)
- Microcentrifuge
- 100% Isopropanol

Protocol:

1). Add 300µL-1mL of plasma into an appropriately sized tube.
2). Add 1/10 input volume of Proteinase K to each sample.
3). Add 1/2 input volume of BL Buffer to each sample. Vortex to mix.
4). Incubate the samples at 56°C for 15 minutes.
5). Allow the tubes to cool down to room temperature for 2 minutes.
6). Add 2X input volume 100% isopropanol. Vortex to mix for 10 seconds.
7). Transfer 750µL of solution to the Binding Column/Collection assembly.
8). Centrifuge the assembly at 10,000 x g for 30 seconds. Discard the flow through.
9). Repeat steps 7-8, until all the solution has been passed through the Binding Column/Collection assembly.
10). Add 500µL of 1X Wash Solution (with ethanol added) to the Binding Column/Collection assembly.
11). Centrifuge at 10,000 x g for 30 seconds. Discard the flow through.
12). Repeat steps 10-11.
13). Centrifuge the binding Column/Collection Tube assembly at 16,000 x g for 3 minutes to dry.
14). Add 50µL of Nuclease-Free Water.
15). Centrifuge at 16,000 x g for 1 minute.

Results:

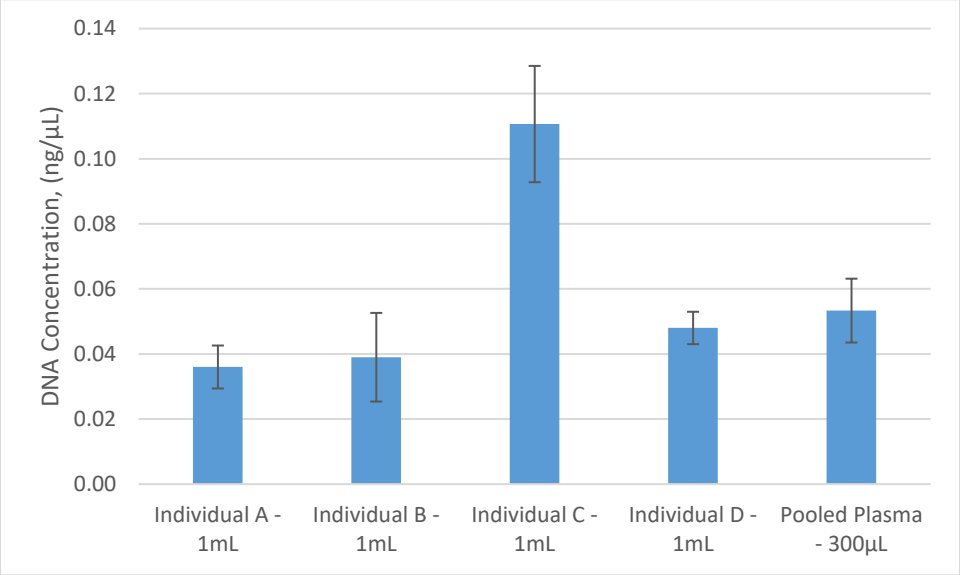

Figure 1. DNA concentration was measured using the 75bp amplicon of the ProNex® DNA QC Assay, for ccfDNA purified from plasma using the ReliaPrep™ FFPE gDNA Miniprep System. ccfDNA was purified from 300µL of pooled plasma and 1mL of individual plasmas in triplicate. Concentration was determined using the 75bp amplicon of the ProNex® DNA QC Assay. Mean ± Standard Deviation of n=3 is shown.

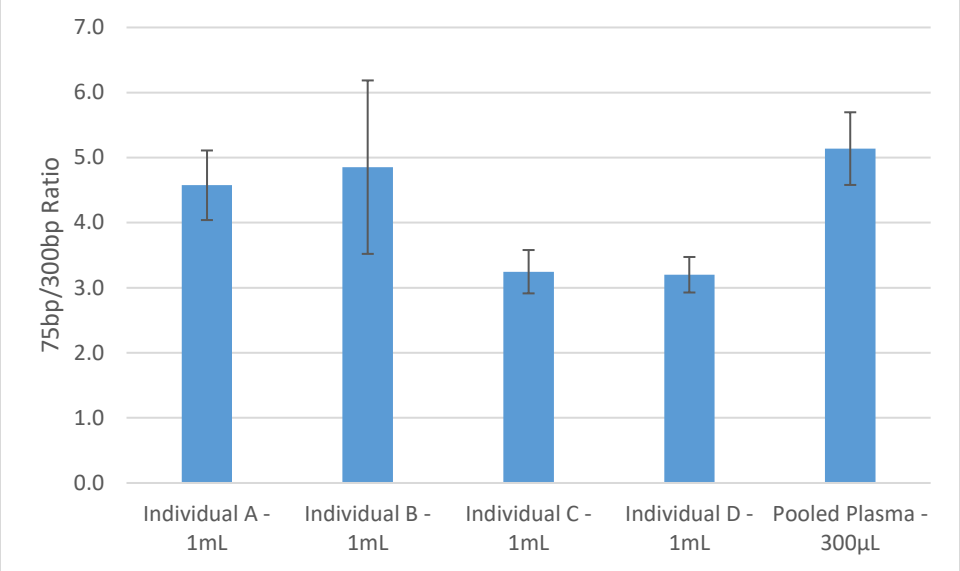

Figure 2. 75bp/300bp DNA ratio was determined using the ProNex® DNA QC Assay for ccfDNA purified from plasma using the ReliaPrep™ FFPE gDNA Miniprep System. ccfDNA was purified from 300µL of pooled plasma and 1mL of individual plasmas in triplicate. 75bp/300bp DNA degradation ratio was determined using the ProNex® DNA AC Assay. Mean ± Standard Deviation of n=3 is shown.