

Concentration of FFPE DNA using the ReliaPrep™ DNA Clean-Up and Concentration System

Concentrate DNA from dilute, high volume FFPE samples using the ReliaPrep™ DNA Clean-Up and Concentration System

Kit: ReliaPrep™ DNA Clean-Up and Concentration System
(Cat.# A2891)

Analyses: QuantiFluor® ONE dsDNA System (E4871) and
ProNex® DNA QC Assay (NG1004)

Sample Type: Purified DNA from FFPE tissues

Input: Up to 300µL

Materials Required:

- Microcentrifuge
- Vortex
- 100% Isopropanol
- 95-100% Ethanol

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.

Further information can be found in Technical Manual #TM540, available at: www.promega.com/protocols

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

Protocol:

Note: ReliaPrep™ DNA Clean-Up and Concentration System is designed for 50-300µL input, and yields 30µL of eluate. Only volumes on the higher end of this range will result in substantial sample concentration.

1. Pipet up to 300µL of dilute DNA into a 1.5mL microcentrifuge tube.
2. Add ½ volume Membrane Binding Solution and vortex for 5 seconds.
3. Add 1 ½ volumes of 100% isopropanol.
4. Load sample onto a ReliaPrep™ Minicolumn seated in a Collection Tube and centrifuge for 30 seconds at 16,000 x g.
5. Remove column, and discard the contents of the Collection Tube. Reseat the column into the same Collection Tube.
6. Add 200µL of Column Wash Solution (CWE) and centrifuge for 15 seconds at 16,000 x g. Remove column, and discard contents of the Collection Tube. Reseat the minicolumn into the same Collection Tube.
7. Wash with 300µL of Buffer B (BWB) and centrifuge for 15 seconds at 16,000 x g. Repeat wash with 300µL of Buffer B (BWB) and centrifuge again at 16,000 x g.
8. Remove column and discard the contents of the Collection Tube. Reseat the column into the same Collection Tube and centrifuge for 1 minute to dry the columns at 16,000 x g; then transfer columns to Elution Tubes.
9. Pipet 15µL of Nuclease-Free Water or TE buffer (not provided) into the center of the ReliaPrep™ Minicolumn and then centrifuge for 30 seconds.
10. For maximum recovery, repeat elution with an additional 15µL of Nuclease-Free Water or TE buffer.

Results:

All DNA samples were purified from 10µm FFPE section of human colon tumor using the Maxwell® RSC FFPE DNA Kit (Cat.# AS1450) with the standard protocol (#TM437). Samples were concentrated in triplicate from 300µL to 30µL using the ReliaPrep™ DNA Clean-Up and Concentration System (Cat.# A2891) following the standard protocol (#TM540).

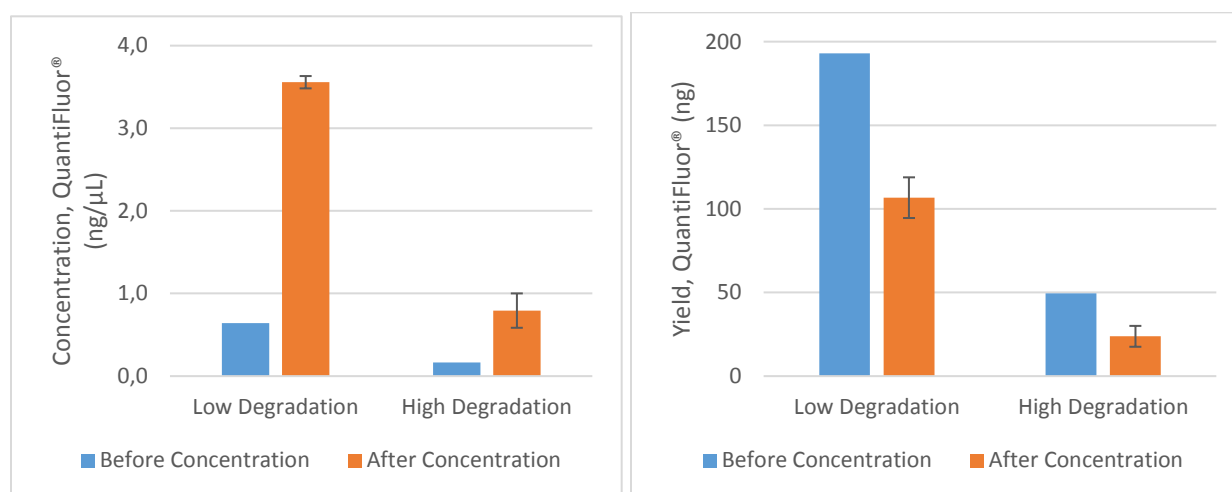


Figure 1. DNA concentration (left) and DNA yield (right) measured using QuantiFluor® ONE dsDNA System (Cat.# E4871) for human FFPE colon tumor DNA with high and low amounts of DNA degradation, concentrated using the ReliaPrep™ DNA Clean-Up and Concentration System (Cat.# A2891). Mean ± Standard Deviation of n=1 for the stocks and n=4 for 300µL of input is shown.

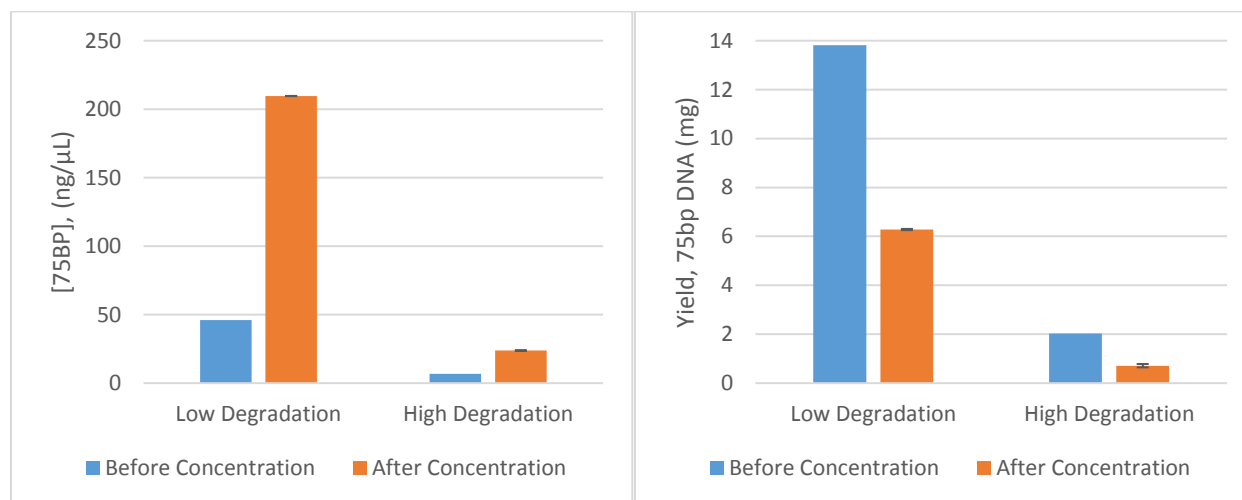


Figure 2. DNA concentration (left) and DNA yield (right) was measured using the 75bp amplicon of the ProNex® DNA QC Assay (Cat.# NG1004) for human FFPE colon tumor DNA with high and low amounts of DNA degradation concentrated using the ReliaPrep™ DNA Clean-Up and Concentration kit (Cat.# A2891). Mean ± Standard Deviation of n=1 for the stocks and n=4 for 300µL of input is shown.