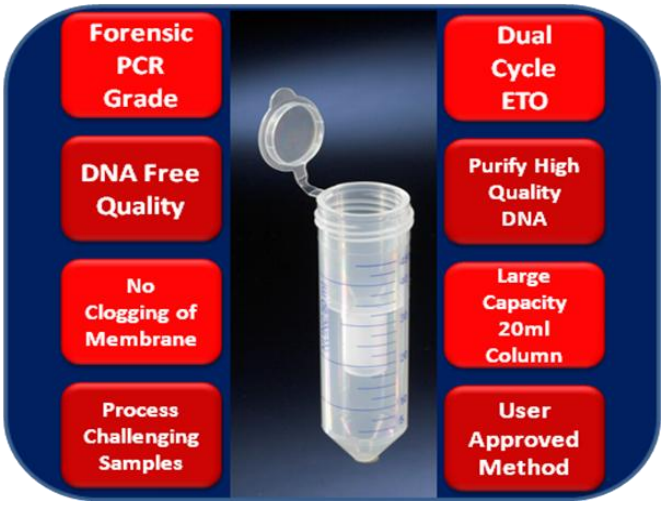
Proteus Hi-Flow® DNA Purification Spin Columns (20 ml)

User Guide

Introduction

Protein Ark Hi-Flow® spin columns offer a novel large volume sample format for the optimal purification and recovery of limited DNA from a variety of difficult samples including forensic casework, human remains and clinical pathology specimens such as bone, teeth and formalin-fixed paraffin embedded material. Hi-Flow® spin columns have a 20 ml capacity, very useful for processing samples with limited DNA and highly efficient purifications are achieved using our dual-cycle ethylene-oxide treated silica membrane device.



Recover purified degraded DNA faster and better with minimal handling of sample – no organic extractions required, just use crude extracts. Special dual cycle ethylene-oxide treatment of the Hi-Flow® spin columns mitigates extraneous contamination issues which is ideal for forensic and human identification labs requiring DNA-free consumables.

Hi-Flow® for DNA – Key Features & Benefits:

- Process large volume, low DNA, highly contaminated samples. Gain better purity.
- Large volume sample purification with 20 ml column capacity - avoid using multiple tubes of smaller aliquots such as micro spin columns thus saving time and potential contamination.
- Simply use crude extract – no organic extraction required. Columns fit 50 ml conical centrifuge tubes.
- Easy to follow purification method - compatible with commonly used high salt DNA purification buffers found in your laboratory.
- Good removal of contaminants and inhibitors for better downstream analysis such as PCR, STR analysis, SNP genotyping, sequencing and more.
- Better purity than ultra-filtration approaches. Elute in small volumes.

- DNA-Free Product suitable for sensitive PCR-Grade DNA applications.
- Special dual-cycle ethylene-oxide treated Hi-Flow spin columns for forensic use – mitigate consumable contamination issues especially in forensic analysis.
- Purification complete in under 2 hours, batch process possible, process many samples in parallel.
- Strengthen your technical process, enhance quality, better your success with Protein Ark Hi-Flow® columns.

Specification:

Plastic spin column construction:	Polypropylene
Membrane composition:	GF Silica grade membrane
Maximum column reservoir volume:	20 ml
Typical g force:	2,600 x *g*
Typical Sample Loading Spin times:	2-10 min
Minimum Elution volume:	50 – 100 µl
Maximum Elution volume:	up to 500 µl
High Mol Wt. DNA Recovery:	>80%
Wide Binding Capacity of Column:	Broad range, from few nanograms to >10 µg
Maximum g force:	3,500 x *g*
Storage:	Store at room temperature
DNA-Free Status:	DNA-Free product, PCR Grade quality
Sterilisation:	Dual Cycle Ethylene oxide gas sterilisation

Additional Materials Required:

This user developed protocol has been optimised for use with well known commercially available buffer sets. It is designed to purify and recover double-stranded DNA from a variety of challenging samples such as bone, soft tissue, clinical samples, plant material or difficult agricultural and veterinary samples.

- Centrifuge accepting 50 ml conical tubes.
- 50 ml centrifuge tubes.
- Chaotropic DNA Binding buffer (e.g. commercially available Qiagen Buffer PB)
- Ethanol Wash buffer (e.g. commercially available Qiagen Buffer PE)
- Low Tris pH 8.5 Elution buffer e.g. commercially available Qiagen Buffer EB
- Sample source: Supernatant of crude DNA extract or pre-lysed material of your choice

Technical support:

Contact the Protein Ark technical support and sales centre for assistance:

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Disclaimer:

- This product is for research use only and is not intended for use in clinical diagnosis. No claims beyond replacement of unacceptable material or refund of purchase price shall be allowed.

Ordering Information:

Product	Units	Order Code
Hi-Flow DNA Purification Spin Columns, 24 Pack	24	GEN-HF24ETO
Hi-Flow DNA Purification Spin Columns, 96 Pack	96	GEN-HF96ETO

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Recommended Protocol:

1. Transfer the sample extract to a 50 ml conical tube and add 5 volumes of high salt binding buffer (e.g. Qiagen Buffer PB). (i.e. 0.5 ml of extracted supernatant would require 2.5 ml of PB buffer, while 3 ml of supernatant would require 15 ml of PB buffer)

2. Vortex briefly.

3. Add the entire sample/PB mixture to a Hi-Flow® column that has been placed in one of the 50 ml conical tubes (as a “catch” tube). This tube is emptied and re-used through step 8.

4. Centrifuge at 2,600 x g for 10 minutes, and then discard flow-through buffer.

5. Add 10 ml ethanol Wash buffer (e.g. Qiagen Buffer PE) to Hi-Flow® column, centrifuge at 2,600 x g for 10 minutes and then discard the flow-through.

6. Optional steps - for particularly poor samples; repeat step 5 two more times using 5 ml Buffer PE, for a total of three washes. Otherwise go to step 7.

7. Centrifuge the "empty" Hi-Flow® column at 2,600 x g for 5 minutes.

NOTE: This is necessary to remove residual alcohol from the column.

8. Transfer Hi-Flow® column to a new 50 ml conical tube.

9. Add 100 µl Elution buffer (e.g. Qiagen Buffer EB, 10 mM Tris-HCl pH 8.5) directly to Hi-Flow® column membrane.

NOTE: You can elute in any low salt solution, including water, but a slightly alkaline solution solubilises the DNA most effectively.

10. Leave at room temperature for 5 minutes to reconstitute the DNA.

11. Centrifuge at 2,600 x g for 2 minutes to collect eluate in the new 50 ml conical tube. Optional: transfer eluate to 1.5 ml tube for storage.

12. Repeat steps 8 through to 11 once again (for a total of two elutions) and keep the eluates separate. Optional: transfer eluate to 1.5 ml tube for storage.

Product Performance:

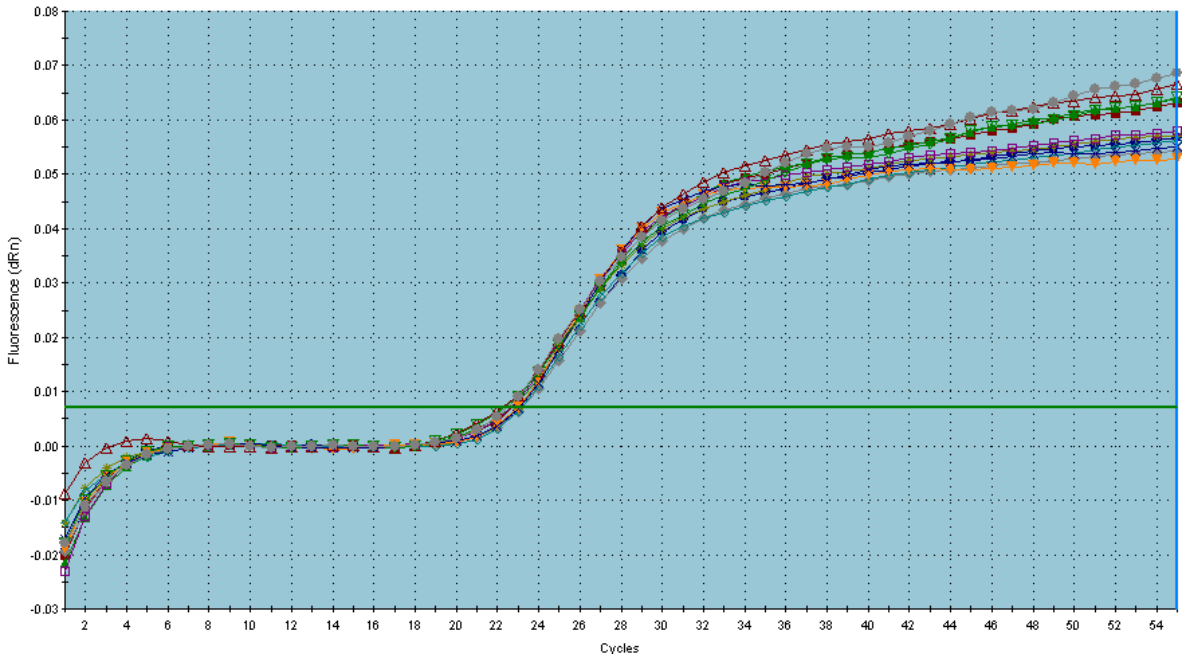

Figure 1: Highly Pure DNA is obtained after Hi-Flow® purification – Reproducibly low variation is observed in real-time PCR cycle threshold (Ct) values of internal control using Taqman probe chemistry.

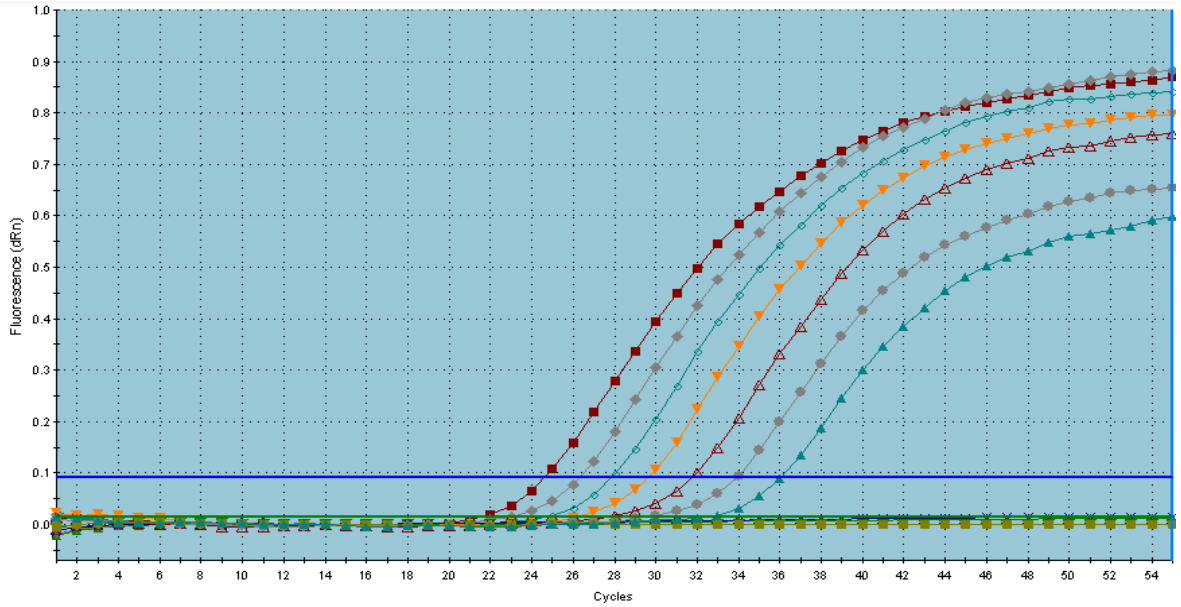

Figure 2: DNA-free results from dual-cycle ethylene-oxide treated Hi-Flow® devices – No human genomic DNA signal was observed after amplifying Hi-Flow® purified DNA for 55 PCR cycles using Taqman probe chemistry (baseline plots). Amplification plots for the DNA standards are shown representing human genomic DNA positive controls.

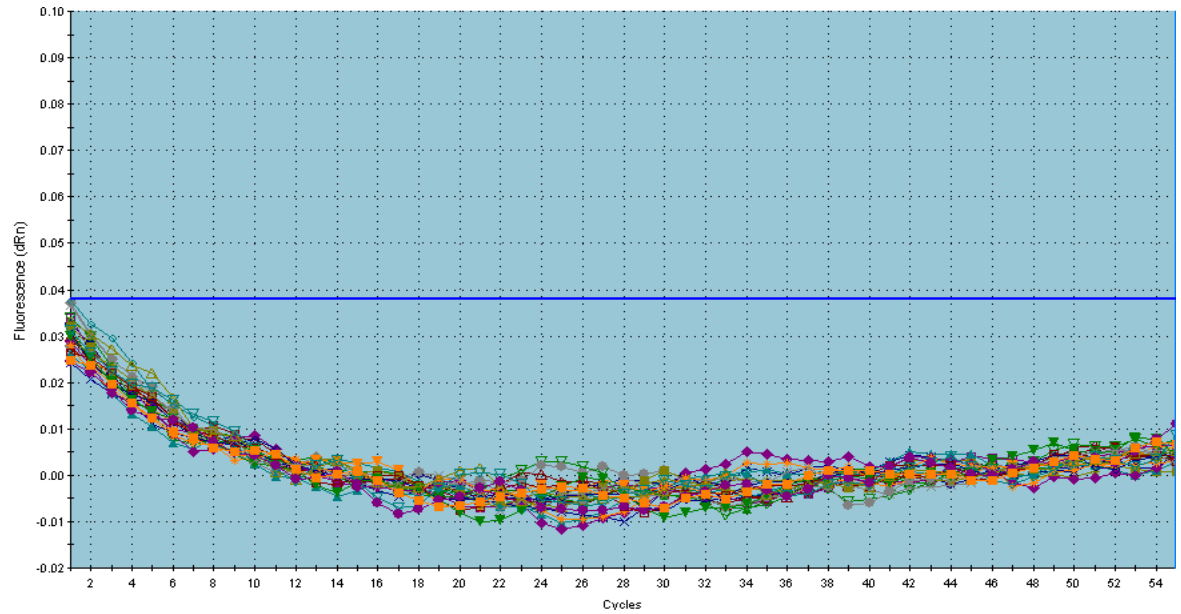

Figure 3: DNA-free results of ethylene oxide-treated (EtO) Hi-Flow® devices – No human genomic DNA signal was observed after amplifying Hi-Flow® eluted samples for 55 PCR cycles using Taqman probe chemistry. Results from non-EtO devices are shown in the same group of amplification plots.

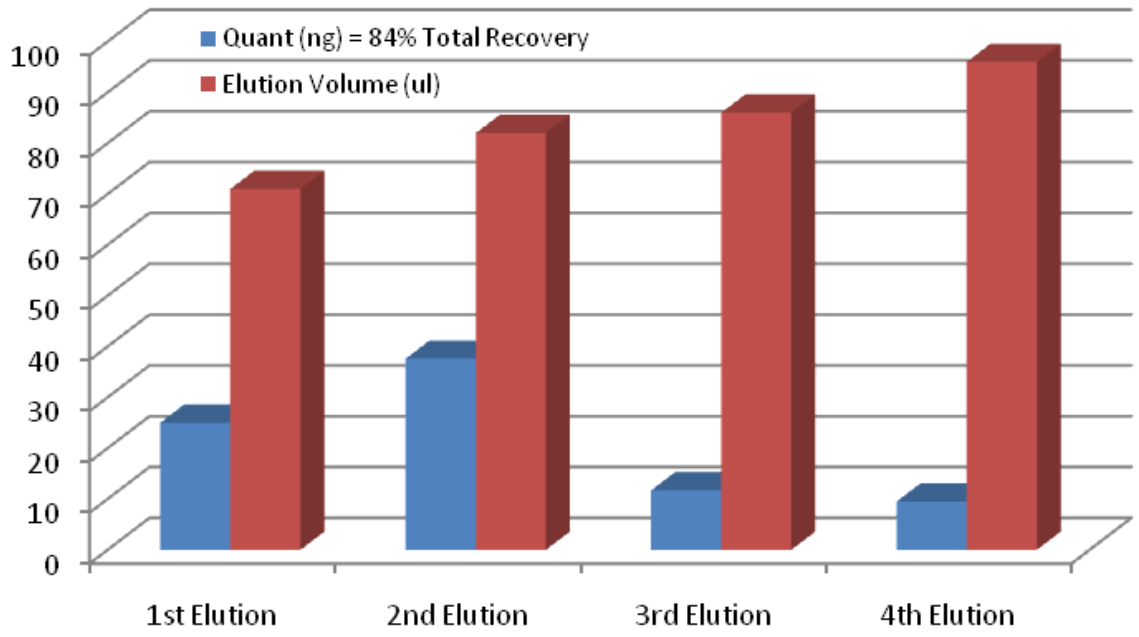

Figure 4: High DNA yield is observed. A starting amount of 100 ng high molecular weight human genomic DNA was purified through EtO-treated Hi-Flow® columns and gave a final recovery of 84% across four sequential elutions.

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