

Quantitation of picogram levels of residual host cellular gDNA from bioprocess samples



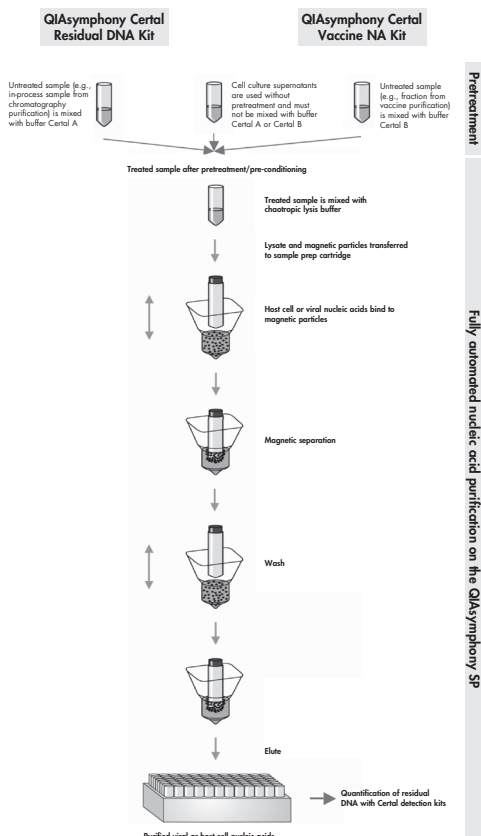
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Introduction

When working with biopharmaceuticals, it is essential to evaluate the final therapeutic material to ensure that it is free of contaminating substances, such as residual DNA from the host cell. Quantitative data is needed to ensure a high quality, safe therapeutic product. The presence of residual host cell DNA in the final product is of concern due to its potential oncogenic properties.

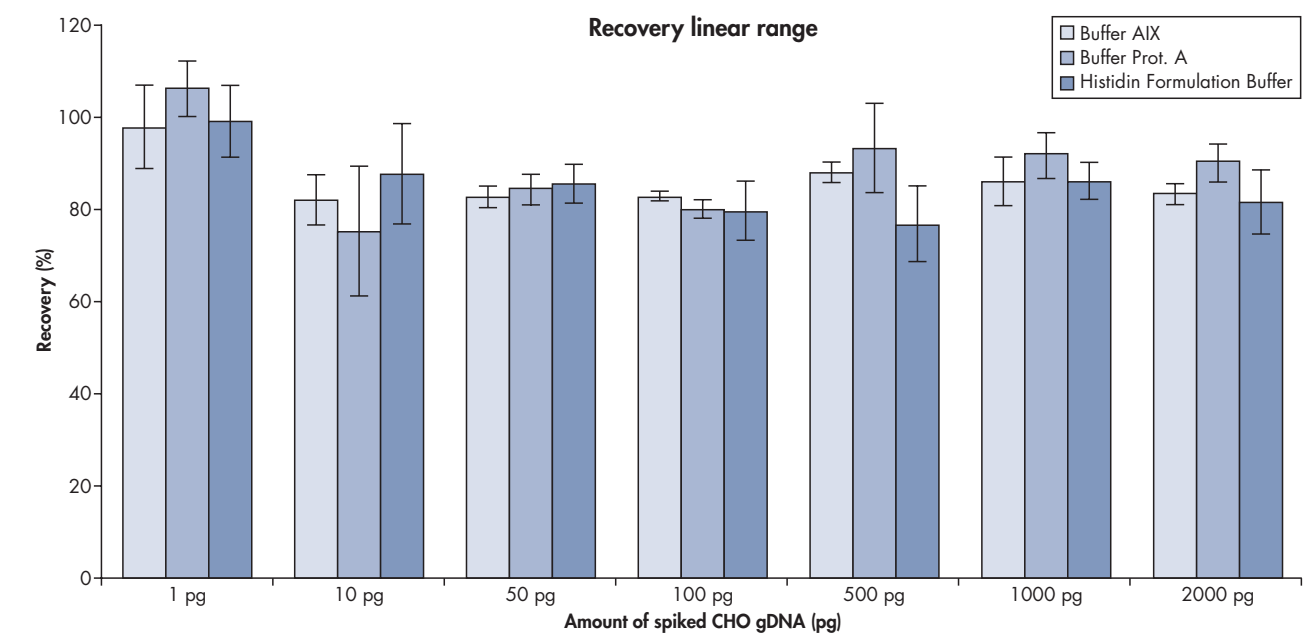
Host cell DNA should be detected using a sensitive analytical technique that yields quantitative data. DNA extraction is a primary concern as the gDNA must be recovered from in-process samples and diverse sample matrices with a consistently high recovery, reproducibility, and robustness. Use of a specially formulated conditioning buffer to equalize samples allows a standardized processing independent of the nature of the matrix sample. The QIAAsymphony® Cortal Residual DNA Kit and Cortal Vaccine NA Kit provide automated solutions for purification of residual host cell DNA and viral nucleic acids from bioprocess purification buffer, cell culture supernatant samples, and vaccine preparations using the QIAAsymphony SP.

Subsequent quantitation of host cell DNA and viral nucleic acids using the Cortal CHO Detection Kit and the Cortal Vero Detection Kit on the QIAAsymphony AS and Rotor-Gene® Q enable evaluation of the quality and safety of biologic medicinal products.



High recovery rate, independent of sample matrix

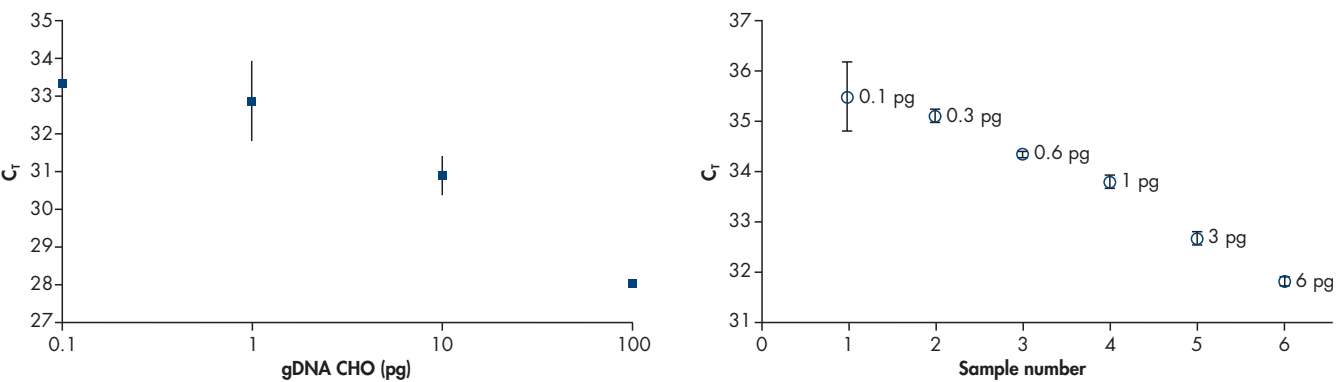
QIAAsymphony Cortal Kits purify residual host cell DNA equally well from a broad spectrum of sample matrices. We formulated a unique conditioning buffer to optimize the recovery rate of residual gDNA, independent of the sample matrix or process buffers with significantly different pH, salt, or protein concentrations. There is no need for extensive pretreatment of different sample types, making final product release testing more straightforward.



gDNA purification from different process buffers. Different amounts of CHO gDNA (1–2000 pg) were spiked into samples with different process buffers [Buffer A: 50 mM NaH₂PO₄, 1M NaCl, pH 7.2; Buffer B: 0.1M glycine, pH 3.2; Buffer C: 16 mM histidine, 266 mM sucrose, 0.03% Tween®20, pH 6.0]. Each process buffer also contained 5 mg/ml hlgG.

Flexible and fully automated purification procedure delivers high precision, down to trace amounts

QIAAsymphony Cortal Kits combine the speed and efficiency of silica-based purification with the convenient handling of magnetic particles. Innovative, ready-to-run reagent cartridges prefilled with magnetic particles and all reagents required for the purification procedure, including accessory enzymes, are automatically opened by the QIAAsymphony SP, minimizing the risk of environmental contamination. Worktable setup is rapid, which saves valuable time. Reagent volumes are calculated only for the selected number of samples, enabling complete cost control. Barcode reading enables full tracking of reagents and samples. QIAAsymphony Cortal Kits, automated on the QIAAsymphony SP, deliver high precision from experiment to experiment and also allow purification of even low (trace) amounts of residual host cell DNA.

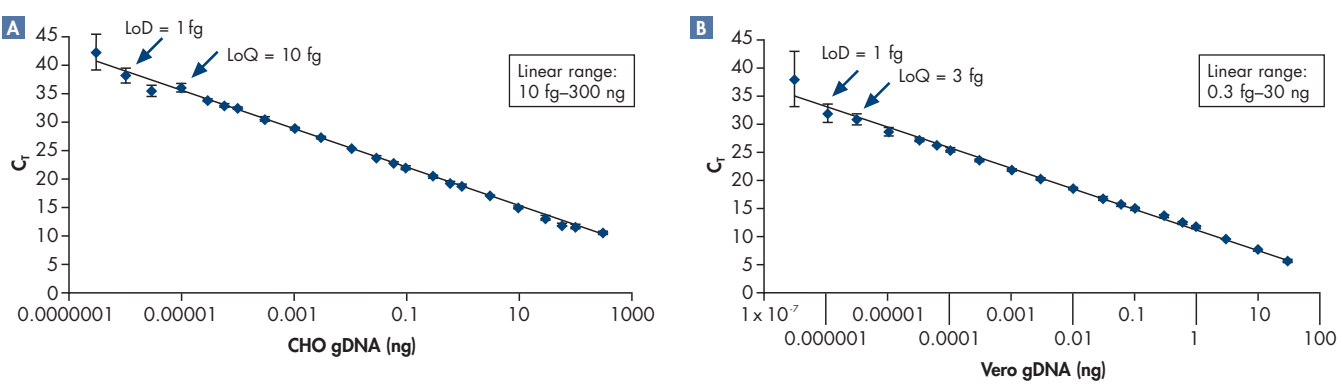


High precision. 500 µl sample from anion-exchange (AIX) chromatography containing different amount of spiked CHO gDNA (0.1–100 pg) were pretreated with 500 µl Cortal A Buffer and applied to the QIAAsymphony Cortal Residual DNA Kit. After extraction of residual gDNA on the QIAAsymphony SP, the amounts of extracted NA were analyzed using the Cortal CHO Detection Kit. Mean C_q and SD over 3 independent runs with 3 different kit lots, 2 instruments and 2 operators is shown.

Lower limit of purification. Genomic CHO DNA was spiked into buffer AEX and 4 replicates purified and determined by qPCR in triplicates. The graph shows the mean of the replicates and SD.

Highly sensitive detection using Cortal Detection Kits for quantitative PCR

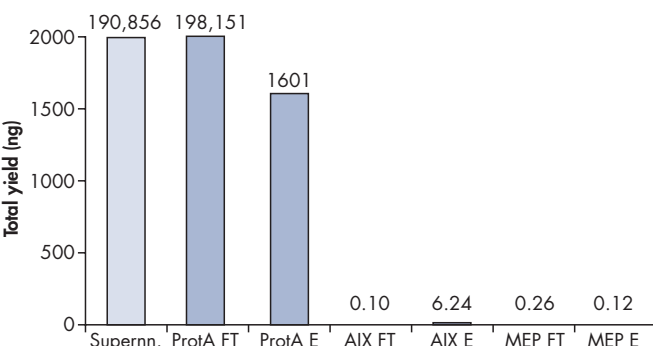
The Cortal CHO Detection Kit and the Cortal Vero Detection Kit enable sensitive and rapid real-time PCR quantification of residual DNA using dual-labeled probes (hydrolysis probes, also known as TaqMan® probes) with a variety of real-time PCR cyclers without optimization. Specific residual host cell DNA is detected with the Cortal Residual DNA Assay, which consists of a premixed primer pair and a hydrolysis probe (FAM™ labeled). The Cortal Internal Control Assay, containing a premixed primer pair and a hydrolysis probe (MAX™ labeled), is for universal use as an amplification control. The Internal Control is detected in the same tube with the residual DNA target in a duplex PCR to test for successful amplification (e.g., exclusion of PCR inhibitors). Limit of quantification (LoQ) and linear range for the CHO and Vero qPCR assay were determined, demonstrating a quantitation limit for gDNA for the whole extraction procedure down to 1 pg residual host cell DNA.



Low limit of quantification. Serial dilutions of **A** CHO genomic DNA (300 ng–0.3 fg) with an LoQ of 10 fg and **B** Vero genomic DNA (30 ng–0.3 fg) with an LoQ of 3 fg were processed in triplicates. LoD: Limit of detection.

Small-scale antibody purification process and analysis of residual DNA clearance

The development process for the production of a biological therapeutic (e.g., a monoclonal antibody) in cell lines includes the need to prove the absence of host cell contaminants (e.g., genomic DNA) and adventitious agents (e.g., cell-line specific viruses) in the final product, and also to demonstrate their removal during the purification process in a clearance study. To demonstrate the sensitive quantitation of our method, we performed a small-scale antibody purification model process.



500 µl of each fraction from small-scale antibody purification process were mixed with 500 µl Buffer Res3 prior to DNA extraction using the QIAAsymphony. The DNA level of each in the in-process samples was measured by qPCR using an assay specific for CHO gDNA.

Process pool	DNA level (ng total)	DNA clearance (log)
Supernatant	190,856	NA
Protein A	1601	2.1
Anion exchange	6.24	2.4
Hydrophobic charge ind.	0.12	1.7
Overall clearance	NA	>6.2

The DNA clearance steps in the process were shown to be Protein A and AIX chromatography steps, with 2.1 and 2.4 log clearance, respectively. Log reduction is the level of DNA clearance from the chromatographic load to the pool expressed as log₁₀. NA: Not applicable.

Conclusions

- Flexible and fully automated purification on the QIAAsymphony enables the user to purify as little as 1 pg host cellular gDNA from a broad variety of bioprocess samples with recoveries of 80–100%.
- Optimized protocols and reagents as part of streamlined workflow ensure consistent, reliable, high-quality results.
- Excellent reproducibility in combination with low limit of quantification.
- Complete kits and proven protocols support user requirements.



The QIAAsymphony RQG.

The applications presented here are for research use only. Not for use in diagnostic procedures.

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