Direct quantification of residual host cell DNA using the QIAcuity® Digital PCR Platform

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Introduction: Host cell DNA contamination

Residual host cell DNA (HCD) monitoring is an important step in the process of manufacturing proteins and vaccines. The potential carryover of HCD poses a safety concern. Levels of HCD must not exceed those established by regulatory agencies such as the U.S. Food and Drug Administration (FDA) and the World Health Organization (WHO). Clear guidelines for the upper limits of residual DNA are established based on the nature of drug administration, infectivity and oncogenity of the contaminating cell DNA. For instance, parenteral administration of non-tumorigenic cell DNA should be limited to 10 ng/dose and maximum length of 200 bp, whereas only less than 100 µg/dose of residual DNA is recommended by WHO for orally administered vaccines.

Detection and removal of such contaminations in manufacturing products requires highly sensitive and accurate measurements of existing extremely low amounts of a specific host cell DNA present in the products.

Digital PCR (dPCR) is the choice of detection for residual DNA quantification as it provides unmatched sensitivity and accuracy of detection at a low template input range and therefore enables a more robust application.

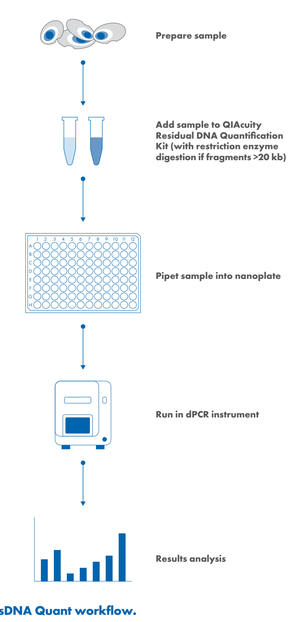
QIAcuity instruments.

Detect carryover of host cell DNA with high accuracy and precision

QIAcuity Residual DNA Quantification Kits together with the QIAcuity Digital PCR System enable:

- Accurate detection of host cell DNA – *E. coli*, Chinese hamster ovary cell (CHO) and human embryonic kidney 293 cells (HEK293) host cell DNA.
- Residual DNA detection down to the low femtogram range
- Detection of host cell DNA in both extracted and unextracted samples
- Species-specific multi-copy target assays for sensitive detection of highly fragmented host cell DNA
- Species-specific dPCR-verified DNA standards and positive control – for validation of quantification accuracy or bridging studies
- Monitoring of PCR efficiency via an internal control
- Easy combination with QIAcuity Nanoplate 26k
- Seamless analysis with QIAcuity dPCR Software
- Flexibility to use on other dPCR platforms

A fast, simple workflow for HCD monitoring



resDNA Quant workflow.

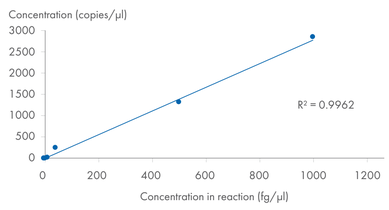
The lysate is added to the resDNA Quant Mastermix together with the internal control and host cell DNA is measured by absolute quantification. Copies/µL is converted to fg/µL with the provided conversion factor.

- Direct sample input – no need for DNA extraction; partitioning minimizes the effects of inhibitors
- Premixed master mix with controls for easy setup and detection of host cell DNA
- No need for a standard curve – dPCR measures absolute copies of target molecules
- dPCR verified DNA standards and controls
- Minimal method development – the resDNA Quant Mastermix is free from detectable levels of HCD
- Multi-copy targets – accurate quantification, even with highly fragmented DNA
- Fast, simple workflow – completed within hours and with easy results analysis
- Flexibility to use on other dPCR platforms

Assay	Target copy number	Amplicon size	Conversion factor cp/µL to fg/µL
QIAcuity *E. coli* resDNA Quant Kit	7	<200 bp	0.35
QIAcuity CHO resDNA Quant Kit	~ 1 million, undefined (repeated element)	<200 bp	0.28
QIAcuity HEK293 resDNA Quant Kit	~ 1 million, undefined (repeated element)	<200 bp	1.54

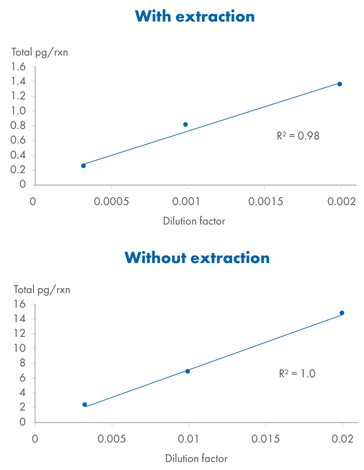
Robust HCD monitoring using QIAcuity resDNA Quant Kits

dPCR offers unmatched accuracy and sensitivity for detection of trace HCD amounts in samples.



DNA target (fg)	DNA concentration in reaction (fg/µl)	Mean value (Copies/µl)	STDEV (Copies/µl)	Coefficient of variation (%)
40000	1000	2851.67	105.24	3.69
20000	500	1329.02	39.75	2.99
4000	40	257.83	9.84	3.82
400	10	26.48	1.21	4.58
40	1	2.53	0.14	5.71
10	0.25	0.69	0.12	16.78
4	0.1	0.32	0.09	28.07

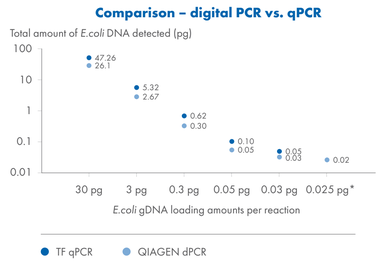
Linear detection using the QIAcuity CHO resDNA Quant Kit. A coefficient of variation ≤20% was obtained down to 40 fg input, confirming a working range of 4000–40 fg DNA input (n=12, triplicates in 4 runs). Reactions were set up according to the standard protocol.



Host cell DNA was checked in cultured media for AAV2 produced in HEK293. The QIAcuity HEK293 resDNA Quant Kit provides a digestion- and purification-free workflow. Samples were tested on the QIAcuity comparing extracted vs. unextracted host cell DNA. Extraction was performed using QIAsymphony® Certal Kits according to the standard protocol. Standard QIAcuity HEK293 ResDNA Quant Kit protocol was used.

dPCR provides higher sensitivity and accuracy compared with qPCR at low sample input range

In contrast to qPCR, partitioning of the bulk sample in dPCR increases the effective concentration of the target, allowing small amount of host cell DNA to be captured and measured within individual partitions with higher accuracy and precision. End-point PCR signals in dPCR allow for detection of target amplicons independent of their amplification efficiency. This increases the sensitivity of detection in presence of PCR inhibitors or contaminants including crude samples without DNA purification. In addition, dPCR detects absolute quantities of target molecules in partitions, eliminating the need for generation of standard curves in qPCR.



Comparison of resDNA kits – dPCR vs. qPCR. Reactions were set up according to the manufacturer's protocol. Results represent averaged values from duplicates. The QIAcuity *E. coli* resDNA Quant Kit (dPCR) provides higher accuracy at low-loading amounts.

*Below dynamic range of TF RNASEQ *E.coli* Quant Kit (qPCR).

Sample name	Template [pg/rxn]	C_T	C_T mean	Ct SD
QIAGEN CHO DNA	0.003	34.045	33.951	0.133
	0.003	33.857		
	0.03	30.769	30.691	0.110
	0.03	30.614		
	0.3	27.475	27.555	0.114
	0.3	27.636		
	3	24.110	24.144	0.048
	3	24.178		
	30	20.696	20.751	0.078
	30	20.806		
Thermo Fisher CHO DNA	0.003	34.738	34.511	0.321
	0.003	34.284		
	0.03	31.262	31.280	0.026
	0.03	31.299		
	0.3	28.265	28.303	0.055
	0.3	28.342		
	3	24.861	24.884	0.032
	3	24.907		
	30	21.430	21.476	0.064
	30	21.521		

Comparison of QIAGEN standard vs. Thermo Fisher standard for CHO with qPCR. qPCR was performed in duplicates. Mean C_T and standard deviation were calculated from duplicates.

Conclusions

QIAcuity Residual DNA Quantification Kits offer precise quantification of host cell DNA in complex bioprocess intermediates, enabling:

- Accurate and precise quantification of residual CHO, *E. coli* and HEK293 host cell DNA
- Absolute quantification of trace amounts of HCD, even in the presence of PCR contaminants and other reagents inhibitory to dPCR
- Multicopy species-specific target assays ensure that results are unaffected by the fragmentation level of the resDNA

These features allow for HCD testing in complex bioprocess intermediates without the need for DNA extraction. QIAcuity Residual DNA Quantification Kits are designed in accordance with the requirements of bioprocess manufacturing and QC process.

Sample to Insight