Introduction: Host cell DNA contamination

Residual host cell DNA (HCD) monitoring is an important step in the processing 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 oncogenicity of the contaminating cell DNA. For instance, parenteral administration of non-tumorigenic cell DNA should be limited to 10 µg/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 unrivalled sensitivity and accuracy of detection at a low template input range and therefore enables a more robust application.

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 (HEK293) host cell DNA
- Residual DNA detection down to the linear threshold range
- Detection of host cell DNA in both naked and extracted samples
- Species-specific multi-copy target assays for sensitive detection of highly fragmented host cell DNA
- Species-specific dPCR-validated 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 Nanoplates 246
- Seamless workflow with QIAcuity dPCR Software
- Flexibility to use on other dPCR platforms

A fast, simple workflow for HCD monitoring

The benefits added to the resDNA Quant Mastermix together with the internal control and host cell DNA is measured by absolute quantification. Copia26S is converted to λ2S with the provided conversion factor:
- Direct input sample – 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 validated DNA standards and controls
- Minimal method development – the resDNA Quant Mastermix is fluorescent detectable levels of HCD
- Multi-copy targets – accurate quantification, even with highly fragmented DNA
- Fast, simple workflow – confirmed within hours and with easy results analysis
- Flexibility to use on other dPCR platforms

Robust HCD monitoring using QIAcuity resDNA Quant Kits

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

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

In contrast, qPCR processing of the bulk sample in qPCR 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 qPCR allow for detection of target DNA independent of the multiplicity or efficiency. This increases the sensitivity of detection in presence of PCR inhibitors or contaminants excluding sample without DNA purification. In addition, dPCR detects absolute quantities of target molecules in partitions, eliminating the need for generation of standard curves in qPCR.

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 inhibitors and other reagents inhibitory to dPCR
- Multi-copy 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 processes.