Detecting residual host cell DNA with Droplet Digital PCR technology

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Ensuring the removal of residual host cell DNA from cell and gene therapy products is crucial for keeping patients safe and avoiding costly consequences. Droplet digital PCR (ddPCR) technology is a sensitive, specific, accurate and easy-to-use technology that delivers reproducible results and analysis for detecting, sizing, and quantifying residual host cell DNA.

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RESIDUAL HOST CELL DNA TESTING

Testing for residual DNA content is a key step in the viral manufacturing process for cell and gene therapy products. Traces of host cell DNA must be removed from cell and gene therapies to avoid oncogenic risks, as well as loss of raw materials and batch products. Regulatory guidelines state that host cell impurities in cell and gene therapies must be limited to <10ng/dose and <200bp/fragment, but common methods, such as qPCR and BioAnalyzer technologies, have low accuracy at those levels.

Detecting residual DNA with ddPCR involves compartmentalizing host cell DNA into droplets. One advantage of this is that samples in complex matrices require no DNA extraction and are directly used in ddPCR after sufficient dilution. This allows close to 100% recovery to be achieved at a 1:75 dilution, saving time and money. These ddPCR assays also show resistance to inhibitors.

HEK293 RESIDUAL DNA TESTING

Bio-Rad offers a variety of kits and a GMP Supermix for detection of residual host cell DNA. This includes residual HEK293 kits for both sizing and quantification. The commonly used method of qPCR for HEK293 residual DNA detection has a cumbersome workflow and low accuracy with high false positive rates. Laboratory developed ddPCR for HEK293 residual DNA detection is usually done on purified samples, increasing workflow burden.

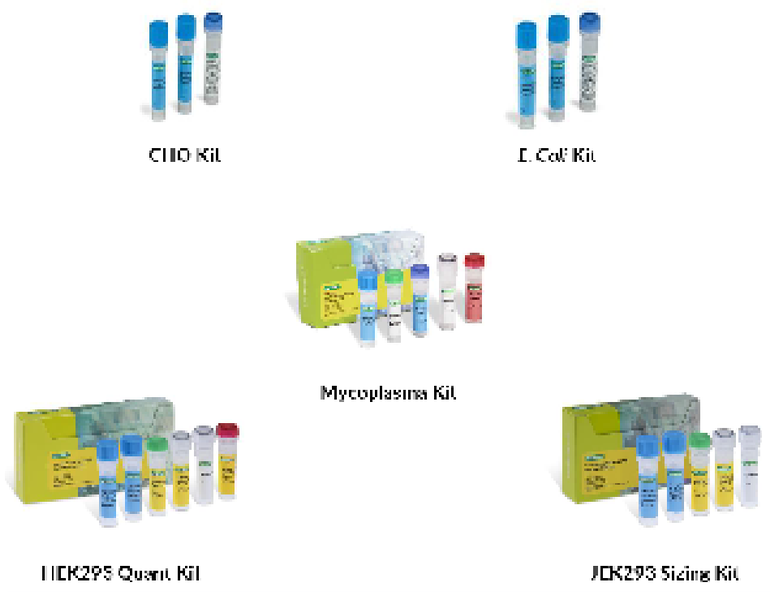
The ddPCR HEK293 residual DNA testing kit from Bio-Rad is the first digital PCR based sizing kit validated to meet regulatory guidance requirements. It provides specific and reproducible sizing solutions, with a sensitivity to quantify HEK293 DNA with LOD of 0.1pg/3 wells. It is easy to use and analyze, as it is an extraction free method. The quant and sizing kits are compatible with AAV and CAR-T workflows. The performance of the quant and size kits is described in **Table 1**. The sizing kit is able to distinguish genomic DNA fragment size of </>200bp. Both kits are validated on AutoDG and QX ONE with a capacity to run 100 reactions.

Table 1. HEK293 kits performance.

Characteristics	Performance metric
***Vericheck ddPCR* HEK293 Residual DNA Quant Kit**	
Sensitivity	0.1 pg /µL (3 wells)
Specificity	99.99% with 4 closely related DNA species
Reproducibility	CV% < 10%
Dynamic range	1pg–80ng at R^2 = 0.998
***Vericheck ddPCR* HEK293 Residual DNA Size Kit**	
Sensitivity	2pg/well (3 well)
Specificity	>99.99% specificity
Reproducibility	CV% < 10%
Dynamic range	2pg–300ng at R^2 = 0.996/0.997

Bio-Rad offers a whole range of ddPCR contaminant testing kits, including a CHO kit, an *E. coli* kit, and a mycoplasma kit, as shown in **Figure 1**.

Figure 1. Manual steps required to perform Ella and ELISA immunoassays.



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