BIO-RAD

Detecting HEK293 Residual DNA Contamination in Your Cell or Gene Therapy Products

Cell and gene therapies begin their development with the help of a host cell line, but before the product can be administered to a patient, any trace of the host cell DNA must be removed to avoid oncogenic effects.

What is HEK293 residual DNA?

HEK293 cells are human embryonic kidney-derived epithelial cells. They are one of the most commonly used cell lines in cell and gene therapy manufacturing. These host cells function as living factories that produce the viral vectors needed to deliver the final therapeutic product. Residual host cell DNA can exist in varying amounts, but being able to precisely quantify how much is crucial to ensuring the safety of the final product. Cell and gene therapy companies must comply with WHO and FDA guidance that states that therapeutics in contact with HEK293 cell lines must not contain residual DNA in excess of 10ng/dose or DNA size distribution of > 200 bp.

Impact of HEK293 residual DNA contamination

HEK293 residual DNA contamination can have serious consequences for patients and biopharmaceutical manufacturers alike, including:

- Oncogenic DNA transfer
- Increased regulatory hurdles
- Delay in therapy delivery
- Loss of raw materials and batch products
- A loss of time and money
- Immune response

Traditional molecular detection methods

Historically, scientists have used these methods to quantify and size host cell DNA:

Sizing: BioAnalyzer

+
- Widely used
- Inexpensive

−
- Not HEK293 specific
- Unable to analyze full-length DNA fragments above 7kb

Quantification: qPCR

+
- Short time to results
- Moderate sensitivitiy

−
- Can produce nonspecific signals
- Requires DNA extraction & standard curves

Despite the strengths listed above, qPCR and BioAnalyzer are limited in their ability to precisely quantify the number of contaminants in a sample and provide data to meet rigorous regulatory standards. Therefore, scientists are continuously developing new technologies and methodologies. One such technology is Droplet Digital™ PCR (ddPCR™).

Introducing ddPCR technology

ddPCR technology is a fast, precise and reproducible molecular detection method for HEK293 residual DNA contamination that is based on water-oil emulsion droplet technology. Compared to other techniques, ddPCR technology provides higher sensitivity and a quantitative readout that reports genome copies per reaction.

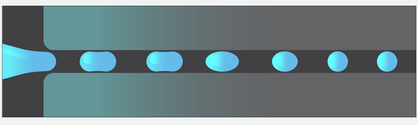
WORKFLOW

1 Prepare ddPCR reaction mix

Combine DNA/RNA sample, primers and probes with a ddPCR supermix.

2 Generate droplets

Load the ddPCR reaction mix into the wells of a droplet generator cartridge. Target DNA and background DNA are randomly distributed into droplets.



3 Perform PCR

Transfer droplets into a PCR plate and run the PCR protocol.

4 Read and analyze results

After PCR, the plates are loaded into a droplet reader. The positive and negative droplets in each sample are read. The concentrations are analyzed with data analysis software.

Have confidence in your testing with BIO-RAD solutions

Bio-Rad's Vericheck ddPCR HEK293 Residual DNA Detection Kits are the first Droplet Digital PCR-based testing solution of their kind, offering:

- High specificity and sensitivity
- Absolute quantification
- Extraction free
- Ability to perform DNA quantification and sizing in one instrument

The QX Manager software can assist in FDA 21 CFR Part 11 compliance when analyzing PCR data, offering:

- A streamlined workflow system control and analysis
- Positive control-based auto-thresholding feature
- Easy data analysis

Learn more about Bio-Rad's Vericheck ddPCR HEK293 Residual DNA Quantification and Sizing Kit Assays

References

1. Wright, J., 2022. Product-Related Impurities in Clinical-Grade Recombinant AAV Vectors: Characterization and Risk Assessment. [online] U.S. National Library of Medicine. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5423478/>.