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Highly Sensitive Nucleic Acid Detection Using qPCR and ddPCR Technology

Polymerase Chain Reaction (PCR) is a fundamental technique in molecular biology, allowing researchers to amplify and quantify nucleic acids such as DNA and mRNA. For biopharmaceutical safety assessment, it is critical to ensure the absence of residual host DNA, such as from CHO or HEK cells used for drug manufacturing. Additionally, with the growing importance of cell and gene therapy (Advanced Therapy Medicinal Products, ATMPs) and mRNA-based drugs, highly sensitive detection methods are essential.

At Eurofins BioPharma Product Testing Munich, we have implemented and validated the most used PCR methods: quantitative PCR (qPCR), also known as real-time PCR, and digital droplet PCR (ddPCR). Both techniques enable DNA/RNA quantification, yet they differ in their principles and applications. While qPCR provides high sensitivity and relative quantification, ddPCR offers absolute quantification without the need for a standard curve, making it ideal for highly sensitive nucleic acid detection.

Technology Principles

Quantitative PCR (qPCR) quantifies nucleic acids by measuring the amplification of a target sequence in real time. This technique typically utilises fluorescent dyes or probes that bind nucleic acids, generating a fluorescence signal proportional to the amount of amplified DNA. By comparing fluorescence levels to a standard curve, qPCR allows for precise estimation of the initial quantity of target DNA with high sensitivity.

Figure 1: Applied Biosystems™ 7500 Real Time PCR system

Digital Droplet PCR (ddPCR) is an advanced PCR method that provides absolute quantification of target DNA without the need for a standard curve. The reaction mixture is partitioned into thousands of tiny droplets, with each droplet functioning as an individual PCR reaction. After amplification, droplets are classified as positive or negative based on the presence of the target nucleic acid thereby allowing for highly accurate absolute quantification of nucleic acids.

Figure 2: Bio-Rad QX200 AutoDG ddPCR System

Automated extraction workflow for qPCR

Before qPCR analysis, manual DNA extraction is a prerequisite but can be time-consuming and limit sample throughput. To enhance efficiency, we have implemented the KingFisher automated DNA extraction system (Thermo Fisher), enabling parallel processing of up to 96 samples while minimising operator-related variability.

Figure 3: Thermo Fisher KingFisher automated DNA extractor

Applications of qPCR and ddPCR

At Eurofins BioPharma Product Testing, we offer a comprehensive range of qPCR and ddPCR services to support various applications:

- Gene Expression Analysis of therapeutic transgenes
- Quantification of residual DNA after in vitro transcription in mRNA drug preparations
- Integrity assessment of mRNA drug substances
- Tandem Quantification of AAV Genome and Transgene Integrity
- Detection and Quantification of residual DNA from CHO or HEK293 cell lines using e.g.:
    - Vericheck ddPCR CHO Residual DNA Quantification Kit (Bio-Rad)
    - Vericheck ddPCR HEK293 Residual DNA Quantification Kit (Bio-Rad)
    - resDNASEQ™ Quantitative qPCR CHO DNA Kits (ThermoFisher Scientific)

Why choose Eurofins BioPharma Product Testing?

qPCR remains the gold standard for rapid and sensitive nucleic acid quantification, while ddPCR provides unmatched accuracy for complex samples and low-abundance targets. Our team of experienced scientists ensures precise and reliable results through state-of-the-art equipment and validated methodologies. We offer personalised solutions and expert support tailored to your specific project needs, ensuring the highest quality standards in biopharmaceutical testing.

Partner with Eurofins BioPharma Product Testing Munich for high-quality qPCR and ddPCR solutions.

Contact Us:

Eurofins BioPharma Product Testing Munich GmbH
Behringstr. 6-8
82152 Planegg/Munich
Germany
pharmatestingbpt.eurofinseu.com