charles river

BIOLOGICS TESTING SOLUTIONS

Quantification of Residual DNA

Residual DNA Quantification

- Methods
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Many cell substrates currently used for recombinant DNA products, monoclonal antibodies and some vaccines are abnormal in that these cells are either tumorigenic or are actually derived from tumors. During the manufacturing of products from these cell culture systems, cell lysis may take place. Therefore, host cell DNA possibly containing an oncogene(s) may be present in the product, which may lead to a tumorigenic event in the recipient. Production schemes generally include purification procedures that remove the nucleic acids; however, it is still necessary to verify the amount of residual host DNA present in intermediate and final product samples.

Methods for Residual DNA Quantification

Charles River uses highly sensitive methods to detect and quantify minute amounts of residual host cell DNA. From the nonspecific detection of total DNA to the detection of species-specific target sequences, we offer the following methods:

Threshold™ assay – The Threshold™ System from Molecular Devices® uses DNA binding proteins which have a high affinity for single-stranded DNA for nonspecific quantification of total DNA.

qPCR – This PCR-based method detects specific DNA of defined origin by targeting a specific gene sequence for amplification. The assay uses an absolute, quantitative standard derived from the appropriate species-matched genomic DNA. Purification of nucleic acid from protein includes a highly efficient system to maximize recovery of very small amounts of DNA using proteases and co-precipitants.

Sample Preparation

Regardless of which method is used for the determination of residual DNA, success of the analysis is heavily dependent on the treatment of the initial sample. Each assay differs in its sensitivity to residuals (e.g., organic solvents, detergents, high salt concentrations, ethanol or residual proteins) and considerations must be taken for a matrix-specific pretreatment. To ensure suitability with the assay and sample matrix, the following pretreatments should be considered: organic extraction, Proteinase K treatment, magnetic beads, phenol-free Wako extraction or other column-based nucleic acid binding methods, and precipitation by ethanol with the addition of co-precipitants. It is important to note that changes in the product purification process affect the sample matrix, which might then affect the assay method.

Assay Validation

According to the International Conference on Harmonisation (ICH) guidelines, all quantitative assays require validation for precision, accuracy, detection and quantitation limits, linearity and specificity. In compliance with ICH guidelines, we validate our DNA assays using a standard matrix, though also perform the sample-specific validations that are required for products at a later clinical stage.