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Quantification of Residual DNA

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 production 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. Purification procedures are generally included in the production scheme to remove the nucleic acids; however, verification of the amount of residual host DNA present is necessary.

Methods for Residual DNA Quantification

Highly sensitive methods exist for detecting and quantifying minute amounts of residual host cell DNA. For nonspecific detection of total DNA to detection of species-specific target sequences, the following methods can be used:

- **Hybridization Assay** – A hybridization-based method for the detection of specific DNA of defined origin using dot blots and hybridization of radioisotope-labeled DNA probes. This method uses random hexamers to generate representative probes which cover the host cells' whole genome or plasmid DNA.
- **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.
- **Q-PCR** – PCR-based method for the detection of 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.

Methods Offered

- Hybridization assay
- Threshold™ assay
- Quantitative PCR

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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 sample requires a matrix-specific pretreatment and all assays differ in their sensitivity to residuals, such as organic solvents, detergents, high salt concentrations, ethanol or residual proteins. Therefore, many DNA purification methods should be tried for their suitability with a specific matrix. Some treatments include: organic extraction, Proteinase K treatment, phenol-free Wako extraction or other column-based nucleic acid binding methods, as well as precipitation by ethanol with the addition of co-precipitants. Keep in mind that changes in the product purification process affect the sample matrix, which might then affect the assay method.

Validation of Assays

Because residual DNA assays are quantitative assays, they must be validated according to International Conference on Harmonisation (ICH) guidelines for precision, accuracy, linearity and specificity. For all intents and purposes, these assays are validated using a standard matrix. A sample-specific validation is required for products at a later clinical stage.