GLYCOTOPE Biotechnology

DNA quantification with the Threshold-System

Principles

Introduction

Following the guidelines (Supplement to the Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology: Nucleic Acid Characterization and Genetic Stability, by the Center for Biologics Evaluation and Research, Food and Drug, Administration, April 1992; CPMP Position Statement on DNA and Host Cell Proteins (HCP) Impurities – Routine Testing versus Validation Studies, by The European Agency for the Evaluation of Medicinal Products, Human Medicines Evaluation Unit, 10 June 1997, CPMP/BWP/382/97) it is necessary to perform routine testing on residual host cell DNA on recombinant products or show in validation studies which steps contribute to which extent in the removal of the DNA burden.

It is assumed that generally the DNA content in a purified product should be below 10 ng DNA per daily dose.

Using the Threshold Total DNA Assay it is possible to perform such studies with high precision and sensitivity (up to 3 pg/ml). In the Threshold Total DNA Assay the detection of DNA is based on the formation of an complex of single-stranded DNA, the single-stranded DNA binding protein (SSB) from *E. coli*, a monoclonal antibody, and streptavidin. The single-stranded DNA binding protein is conjugated with biotin, which strongly binds to streptavidin. The monoclonal antibody is directed against single-stranded DNA and conjugated to the enzyme urease. This complex is filtered through a biotinylated membrane, where it is retained by binding to the membrane-biotin with one of the free binding sites of streptavidin. The urease activity is determined

by the change of the pH at the measurement chamber. The pH change induces on the silicon sensor a change of the surface potential. This change of surface potential is proportional to the pH change. The rate of pH change is proportional to the amount of enzyme and therewith the amount of DNA.

Principles of the measurement:

In the Threshold Total DNA Assay the detection of DNA is based on the formation of an complex of single-stranded DNA, the single-stranded DNA binding protein (SSB) from *E. coli*, a monoclonal antibody, and streptavidin. The single-stranded DNA binding protein is conjugated with biotin, which strongly binds to streptavidin. The monoclonal antibody is directed against single-stranded DNA and conjugated to the enzyme urease. This complex is filtered through a biotinylated membrane, where it is retained by binding to the membrane-biotin with one of the free binding sites of streptavidin. The urease activity is determined by the change of the pH at the measurement chamber. The pH change induces on the silicon sensor a change of the

surface potential. This change of surface potential is proportional to the pH change. The rate of pH change is proportional to the amount of enzyme and therewith the amount of DNA.

Requirements for samples:

- The method is valid between 1.5 to 200 pg DNA in 500 µl solution.
- The pH of the solution has to be between 6.8 and 8.0.
- The ionic strength must be between 100 and 300 mM.
- The sample volume of a single measurement is 500 µl.
- Experience exists about influence and maximal acceptable concentration for many components (on request).

Further sample treatment:

- Samples with a DNA content above 400 pg/ml must be diluted.
- Samples, where DNA concentrations lower than 3 pg/ml should be quantified, have to be concentrated (e.g., by ultrafiltration).
- If the ionic strength is beyond the valid range or low-molecular-weight inhibitory substances (e.g., >20 mM $MgCl_2$) are present, it is recommended to concentrate the samples by microconcentrators (ultrafiltration) and redilute with Zero Calibrator to the desired ionic strength.
- Many proteineous samples can be measured directly. In some cases it is necessary to digest the proteins with proteinase K. Sometimes the mixture has to be further purified by phenol extraction.

Validation of the Assay

After establishment of an appropriate sample treatment, a validation study has to be performed to determine limit of detection, limit of quantitation, accuracy, precision, and robustness within the sample specific setting.