分光光度计的使用

With the aid of spectroscopy, the quantitative analysis of nucleic acids and proteins has established itself as a routine method in many laboratories. It includes absorption measurements in the ultraviolet and in the visibility range. Proteins are measured (directly) at 280 nm, nucleic acids at 260 nm and colorimetric protein determination is carried out in the range from 550 to 600 nm. The BioPhotometer offers the following pre-installed test procedures:

1. Nucleic acid determination

DNA, RNA, oligonucleotides and even mononucleotides can be measured directly in aqueous solutions in a diluted or undiluted form. Aqueous buffers with low ion concentrations (e.g. TE buffer) are ideal for this method. The concentration is determined by measuring at 260 nm against blank and then evaluating via factor. Normally, the user has to calculate the concentration of the measured sample using according factors. In contrast, the BioPhotometer can change these factors easily and will do all necessary calculations.

The absorption of 1 OD (A) is equivalent to approximately 50 µg/ml dsDNA, approximately 33 µg/ml ssDNA, 40 µg/ml RNA or approximately 30 µg/ml for oligonucleotides. Purity determination of DNA Interference by contaminants can be recognized by the calculation of “ratio”. The ratio A260/A280 is used to estimate the purity of nucleic acid, since proteins absorb at 280 nm. Pure DNA should have a ratio of approximately 1.8, whereas pure RNA should give a value of approximately 2.0. Absorption at 230 nm reflects contamination of the sample by substances such as carbohydrates, peptides, phenols or aromatic compounds. In the case of pure samples, the ratio A260/A230 should be approximately 2.2.

2. Protein determination

The protein content of a preparation can be determined on the basis of various different analytical procedures. Evaluation can be carried out via factor or via a calibration curve, with up to ten standards in the BioPhotometer.

Absorption measurement at 280 nm (A280)

A280 method may be used with in concentrations of up to approximately 4 mg/ml

(3.0 A). This method is simple and rapid, but may be disturbed by the parallel absorption of non-proteins (e.g. DNA). Unlike the colorimetric process, this method is less sensitive and requires higher protein concentrations and should thus be used with pure protein solutions. In addition to the direct absorbance display, evaluation is possible with the BioPhotometer via the Warburg formula or via standard.

Colorimetric determination (dye tests)

Protein samples often consist of a complex mixture of many different proteins. The quantitative detection of the protein content is usually achieved on the basis of the reactions shown by functional groups of the proteins to dye-forming reagents. The intensity of the dye correlates directly with the concentration of the reacting groups and can be measured exactly.

Lowry assay 595nm

Specialist literature contains a multitude of modifications for the Lowry assay. The principal target is to reduce the high susceptibility to interference. In comparison to the pure Biuret assay, the sensitivity of this assay has greatly increased. However, the Lowry method is adversely affected by a wide range of non-proteins. Additives such as EDTA, ammonia sulfate or Triton X-100 in particular are incompatible with the test.

Bicinchoninine acid assay 562nm (BCA)

This test represents a highly regarded alternative to the Lowry assay. It is easier to carry out and sensitivity can be varied using different temperatures. Furthermore, the dye complex is very stable. However, this test is highly susceptible to interference, although on the positive side, its insensitivity to detergents is similar to that of the Lowry method.

Bradford assay 595nm

This method is twice as sensitive as the Lowry or BCA test and is thus the most sensitive quantitative dye assay. It is the easiest to handle and most rapid method and has the additional advantage that a series of reducing substances (e.g. DTT and mercaptoethanol), which interfere with the Lowry or BCA test, have no adverse effect on results.However, it is sensitive to detergents. The main disadvantage is that

identical amounts of different standard proteins can cause considerable differences in the resulting absorption coefficients.

3. Bacterial cell density

The density of bacterial suspensions may be measured photometrically at 595 nm without dyes having to be added. This applies e.g. to the preparation of competent cells (i.e. cells, which are able to absorb plasmid DNA), that must be in a specific phase of growth.