

Introduction

The blank measurement as the first step for all photometric measurements is the most critical part for accurate and reproducible sample readings. It is a prerequisite for all photometric measurements.

This Technical Note gives useful information about the blank solution, blank quality and information about the unique Blank Control™ feature in the NPOS software.

Blank Measurement

Blank measurements are reference measurements of the buffer the sample is solved in. Best case the buffer does not absorb light at all, but at least, there should be no absorbance at the wavelength used for the sample measurement e.g. 260 nm for nucleic acids or 280 nm for protein measurements. Due to the blank measurement, the absorbance of the buffer will be recorded and set to zero. For the following sample measurement (buffer and substance of interest) the absorbance change compared to the blank measurement is determined.

Therefore, a poor blank measurement influences the sample measurement directly and may result in too low or too high results.

Quality of Blank Solution and Blank

There are easy and quick options available to check the quality of the blank solution and of the blank. Protein buffers like RIPA, for example, absorb at 280 nm (Figure 1).

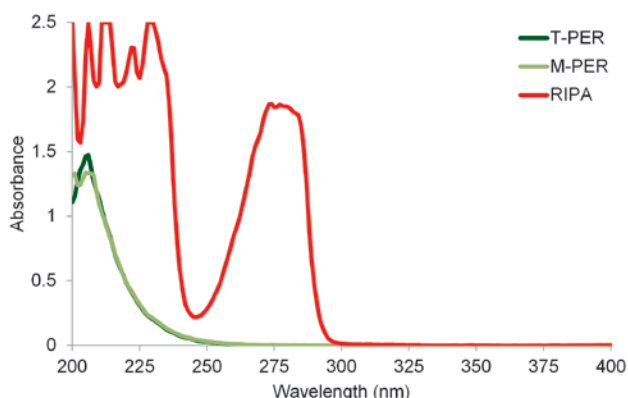


Figure 1: Absorbance spectra of different protein buffers (Blank = dH₂O)

Other protein buffers like T-PER or M-PER show no absorbance at 280 nm (Figure 1). If there are doubts about the used buffer, check the absorbance spectrum by blanking with distilled water and measure the buffer as a sample.

It is recommended to control each blank by remeasuring the blank solution (buffer/distilled water). The control measurement should show a flat line.

Automatic Blank Control™

The NPOS software is analyzing the blank measurement for high background in the wavelength range of 250 – 700 nm and gives the user a warning message (Figure 2) if an absorbance (starting at ~ 2.3 A 10 mm path) is detected. The total range of 250 – 700 nm is split into seven sections (250 – 280 nm, 280 – 340 nm, 340 – 400 nm, 400 – 475 nm, 475 – 550 nm, 550 – 625 nm and 625 – 700 nm) to show at what range the absorbance is appearing.

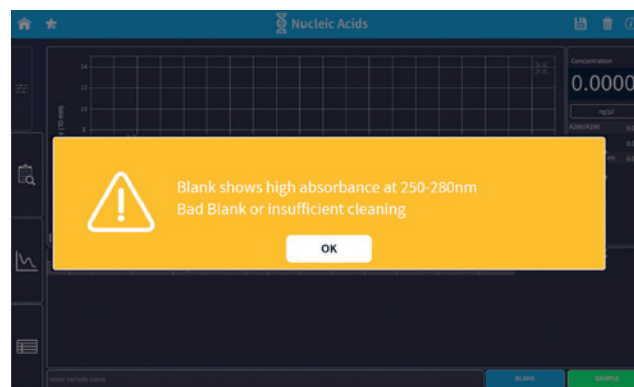


Figure 2: Blank warning message because of high absorbance in the range of 250 – 280 nm

A high absorbance in a blank measurement can have two reasons: either the blank solution/buffer has an absorbance in this wavelength range or the pedestal was not cleaned properly after the last reading.

Conclusion

Implen's unique Blank Control™ will protect you from wasting time and precious sample on inaccurate readings caused by high background blanks or inappropriate cleaning.