Reading an Accurate Blank – The Most Important Measurement of the Day

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Blanking an instrument is a frequent process carried out by researchers in a wet lab numerous times and yet – the comprehension of what reading a Blank on an Implen NanoPhotometer® or any other high quality UV/Vis spectrophotometer does and how easy the Blank reading can be compromised with a significant impact on workflow and results is not really evident to most users.

A Blank reading is actually the most important measurement of the day. If a Blank is inaccurate or goes wrong, all readings following that Blank throughout the day will be incorrect. I would therefore like to introduce the scientific background of a Blank reading and shed some light on factors that can negatively influence the results.

The Lambert Beer's Law[1]

Every time a measurement is taken on a spectrophotometer the light intensity per wavelength is recorded by the instrument and the Transmission (T) is determined:

$$T = I / I_o$$

where I is the light intensity after it passes through the sample and (Io) is the initial light intensity. The relation between (A) and (T) is:

$$A = -\log T = -\log (I / I_o)$$

With every Blank Reading the Transmission of the Blank solution is recorded and stored in the computer of your spectrophotometer as the background for the correction of your sample readings.

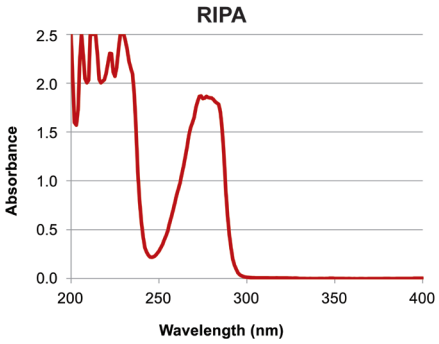
$$A = -\log T = -\log (I / I_o)_{sample} / (I / I_o)_{blank} = e \cdot c \cdot l$$

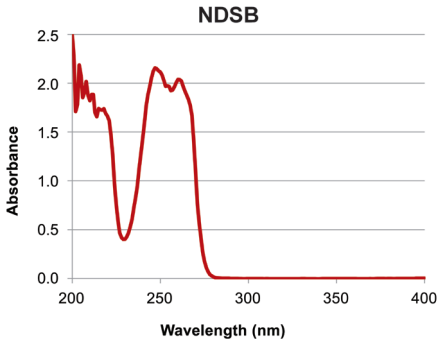
(e) extinction coefficient (c) concentration (l) pathlength

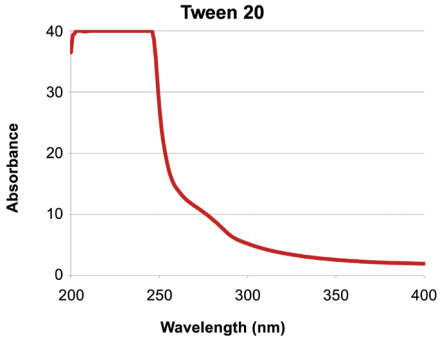
It becomes very obvious that the measured absorbance of a Sample is strongly depending on the background (Blank reading) stored in the system.

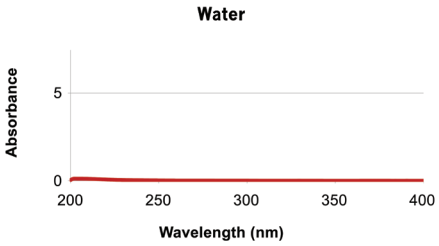
I would now like to outline a few steps that will help a researcher to avoid ruining a full day of hard work just because a Blank was not an accurate Blank! Remember to use a new pipette tip for each step:

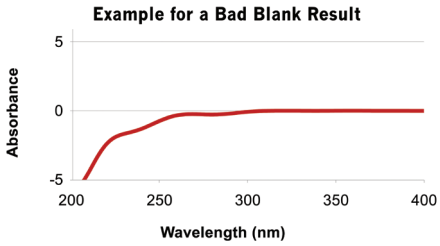
- Always utilize fresh deionized water first to blank the system. Pipette a drop (1 – 1.5 µl) onto the measurement window and take a Blank (in general it is recommended to reverse pipette[2] to avoid air bubbles). If deionized water is not available, a freshly opened bottle of regular drinking water can be used instead. Do NOT use water from open containers even if they are declared RNAse free since algae and different type fungus begin to grow in water once the container has been opened.
- Remove the water after the Blank with a clean, dry Kimwipe™ and reapply fresh deionized water to run it as a Sample this time (again it is recommended to reverse pipette in order to avoid air bubbles). If negative peaks appear or if the scan is not a horizontal flat line, this indicates that either your measurement head was not sufficiently cleaned, or the water used for the Blank has been contaminated. Repeat the Blank until you obtain a completely flat water scan.
- Apply your buffer solution and measure it as a Sample. Again it is recommended to utilize reverse pipetting (buffer solutions commonly contain either detergents or other components known to generate foam. Reverse pipetting is considered mandatory when utilizing buffer solutions containing detergents or other foam generating components). If your buffer solution contains components absorbing within the selected wavelength range, you will immediately see one or multiple peaks. Make sure that these peaks do not coincide with the area of interest for your samples. Some examples of buffers or substances not suitable for use with Protein/Nucleic Acid UV measurements are: RIPA, Tween 20, NDSB, Triton X.
- Some buffers may perish or change with time (e.g. pH modifications). Therefore only use fresh and non-contaminated solutions and make sure that you are not changing the properties of buffers during your sample workflow. Changing pH, alternating EDTA concentrations, etc. will affect your readings and therefore the accuracy of your results (total concentration and ratio readings).
- Once it is verified that the buffer does not interfere with your samples, you may record the buffer solution as a Blank in order to compensate for buffer contributions. Simply apply your Buffer and press Blank. Reapply a new Buffer drop and measure it as a Sample reading to make sure that you receive a horizontal flat line.











Proper cleaning is best done with a moistened Kimwipe™ first and then with a dry wipe to remove remaining moisture from the measurement window and the mirror in the lid. If necessary, a 70/30 alcohol/water solution can be used to remove contaminants not soluble in water. A water moistened wipe and then a dry wipe should always follow cleaning with alcohol to make sure no alcohol remains on the surfaces as alcohol can show absorbance in the higher UV range. Remember to always use fresh deionized water to avoid accidentally introducing contaminants through the back door.

About Reverse Pipetting[2]: Reverse pipetting is a technique generally recommended to dispense solutions with a tendency to foam as it reduces foam or bubble formation. In comparison to forward pipetting, reverse pipetting is much more precise when dispensing small volumes of liquids containing proteins, nucleic acids or any other solutions. To reverse pipette please follow the workflow described below:

1. Make sure the correct tip is attached to the pipette. Push the plunger all the way to the second stop. Put the tip into the sample vessel deep enough to ensure sufficient volume is available to be aspired.
2. Slowly release the plunger until it is back in the original starting position.
3. Dispense the liquid by gently pressing the plunger to the first stop, some liquid including potential foam and air bubbles will remain inside the tip.
4. You may return any left-over sample in the tip back into the sample vessel if desired.
5. Eject the tip.

The Implen NanoPhotometer® is equipped with Blank Control™, a novel technology that will help to identify potential contaminations as well as potential background of a buffer automatically (https://www.implen.de/wp-content/uploads/docs/3_TechnicalNote_Blank_Control.pdf). Still it is recommended to manually secure a good Blank following the protocol described in this publication, especially for the determination of ultra-low concentration samples.

References

[1] UV-VIS-Spektroskopie und ihre Anwendungen, Heinz-Helmut Perkampus, *Springer 1986*

[2] Practical Approaches to Method Validation and Essential Instrument Qualification, Chung Chow Chan, Herman Lam, Xue-Ming Zhang, *Wiley 2010*

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