Generation of a Standard Curve Based Custom Method Using NanoDrop 2000/2000c: Lowry Assay

Key Words
Customized Assays, Lowry Protein Assay, Method Editor Software, NanoDrop 2000/2000c Spectrophotometer, UV Visible Spectrophotometry

Introduction

The Thermo Scientific™ NanoDrop™ 2000/2000c spectrophotometer offers a variety of pre-defined methods such as nucleic acid A260, protein A280, and protein colorimetric assays, but for those who wish to create their own methods, the Method Editor provides step by step customization. Using the Method Editor affords users the ability to tailor custom methods to a particular wavelength range, analysis wavelength, and quantification type using either extinction coefficients or standard curves. In addition to the general quantification parameters, further data analysis can be performed within a custom method by establishing user defined formulas if desired. Custom methods are displayed on the main menu where they can be launched within a matter of seconds.

The Lowry protein quantification method is a colorimetric assay, requiring a standard curve to measure the concentrations of unknown protein samples.[1] The Folin-Ciocalteu reagent used in Lowry assays reacts with proteins, resulting in a product with a broad absorbance range. The NanoDrop 2000/2000c software comes with a pre-defined Lowry assay module, which uses an analysis wavelength of 650 nm, however the broadness of the assay absorbance range means that analysis may be performed at other wavelengths. In order to demonstrate the versatility of the Method Editor software, this paper describes the creation of a custom method designed for use in a situation where the user desires to perform a Lowry protein assay at a wavelength other that 650 nm.

Necessary Information Before Starting

Establishing a custom method requires some prior knowledge about the assay in order to configure parameters, for example, analysis wavelength, quantification type, and spectral correction. Users who are familiar with their assay are able to directly input these parameters by stepping through the Method Editor application and saving the method for future use. On the other hand, users who are not performing standard assays with readily available parameters will need to obtain the pertinent information by measuring the absorbance spectra of the sample. To do this, the first step is to capture full spectrum measurement of a typical sample, in our example a 1 mg/mL protein solution, pre-incubated with Lowry reagent for 30 minutes. At the software startup screen, select the UV-Vis module, follow the instructions to make a blank measurement using deionized water, and remove the water

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sample with a dry lab wipe once the blank is complete. In addition, ensure that the baseline correction is deselected and input 500 nm into the "add wavelength" area as a placeholder. Pipette 2 µL of the developed Lowry sample onto the lower pedestal, lower the arm and select "Measure" to obtain the preliminary spectra of the Lowry sample.

A spectral hallmark of the Lowry assay is the transition from the unbound dye peak at approximately 275 nm sloping down to a trough at the 405 nm and increasing in signal, due to the protein-dye complex, to the broad analysis peak spanning from 650 to 750 nm (Figure 1). After inspection of the spectral output from the UV-Vis module (Figure 1) it can be determined that the spectral correction wavelength is best set at 405 nm as it has the least amount of signal produced by the reaction. The broadness of the absorbance range means that the analysis wavelength need not be set to 650 nm. Instead, in this method, a higher wavelength of 750 nm is used to monitor the signal produced by the Lowry reaction. Finally, the measurement wavelength range for the assay is 350-840 nm, as this wavelength range captures the entire analysis peak. Now that the information required for the Lowry custom method has been obtained, a custom method can be set up in a few easy steps.

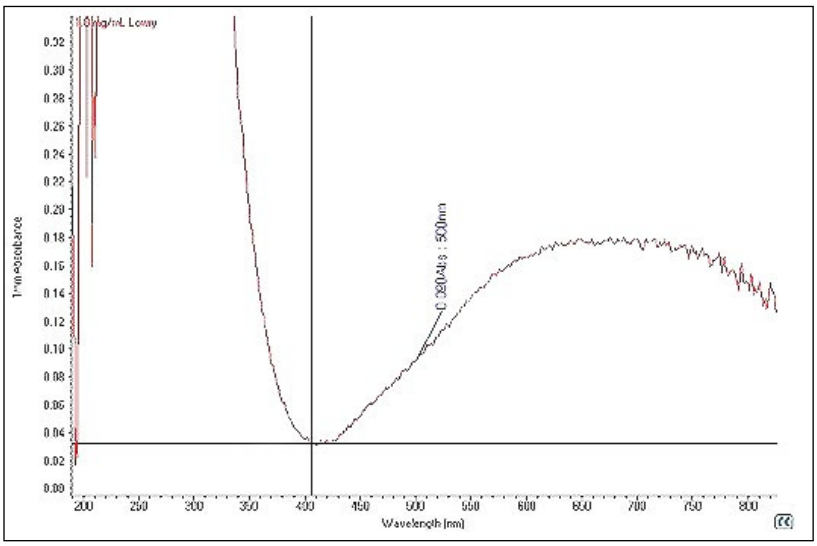
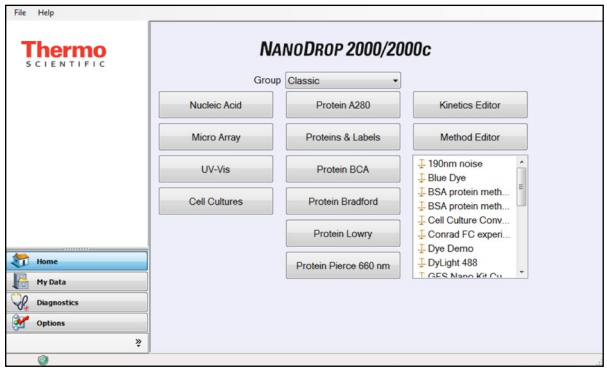


Figure 1: Absorbance spectrum of a developed Lowry sample.

Writing the Custom Method

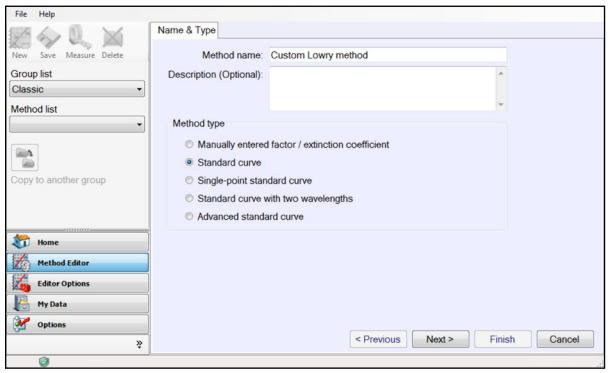
Step 1: Loading the Method Editor

After loading the software, click on the Method Editor button on the main menu.



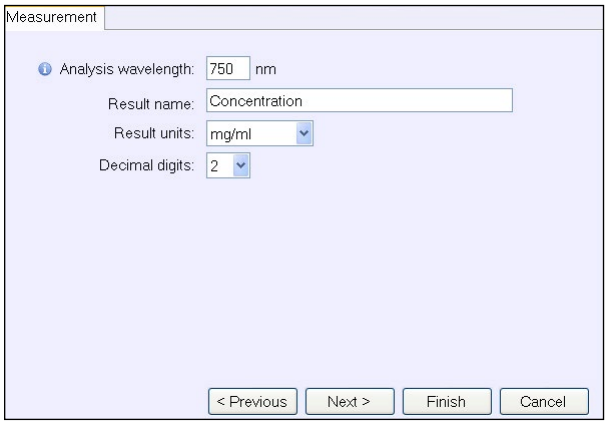
Step 2: Name and Type

After naming the new method, choose the Method type. In this case a standard curve is necessary because the relationship between protein sample concentration and resultant absorbance after incubation with reagents is not linear. For more information on selecting appropriate method type, refer to the user manual.



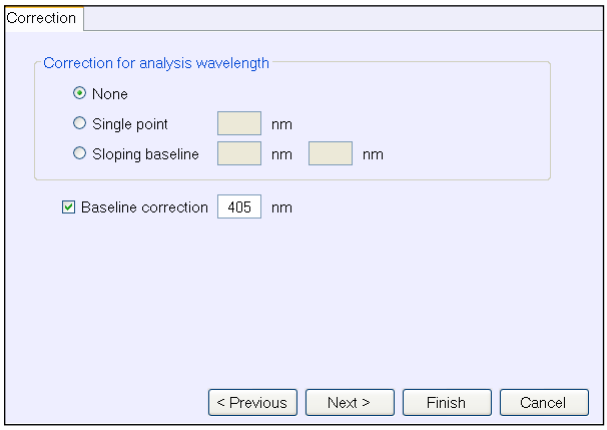
Step 3: Measurement Parameters

Input the analysis wavelength: in this case 750 nm was identified using the earlier UV-Vis measurement. Result name and units depend on your selection of standards; in this example the standards will be varying concentrations in mg/mL. The number of decimal digits used for results may be set as desired.



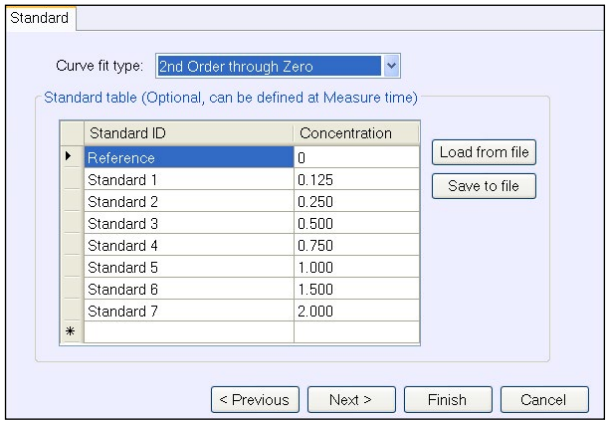
Step 4: Spectral Correction

The use of a baseline correction is highly recommended in order to remove any artifact signal from light scattering produced by sample turbidity. In this case, the UV-Vis measurement showed 405 nm to be a suitable wavelength. The correction for analysis wavelength is set to "None" as there is no need to perform a secondary correction to the measured absorbance value based on absorbance at an additional wavelength. For more information on correcting the analysis wavelength, refer to the user manual.



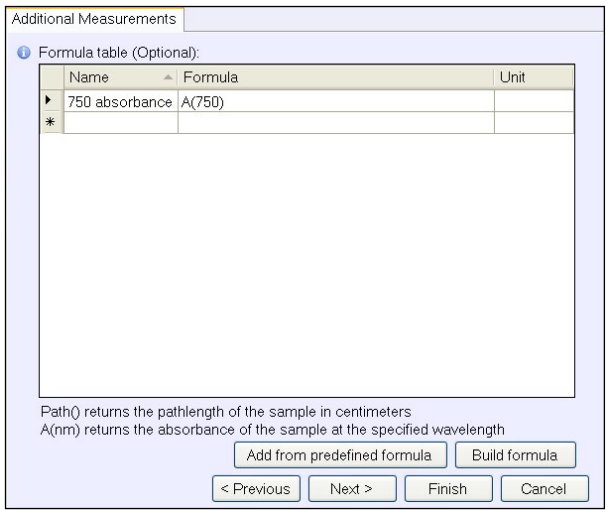
Step 5: Standards

Enter standard curve values (use a wide enough range to encompass all possible sample concentrations). Select curve fit type; in the case of Lowry this should be 2nd order through zero. The curve fit type and concentration range for standards may have to be empirically determined for new assays. The curve fit type can be established by measuring the absorbance of a suitably wide protein concentration range (e.g., Figure 3).



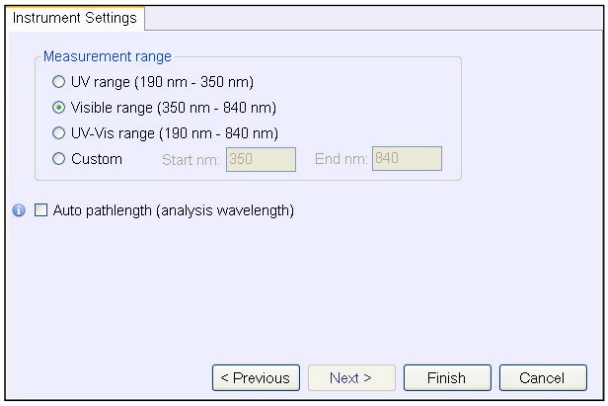
Step 6: Additional Measurements

Enter custom formulas or the absorbance of other wavelengths to interest. Although in this case the method will generate concentration data based on the standard curve, many users also wish to record the actual absorbance at the analysis wavelength of each sample. Entering the formula "A(750)" as shown here means that this absorbance data will be recorded in addition to the concentration data.



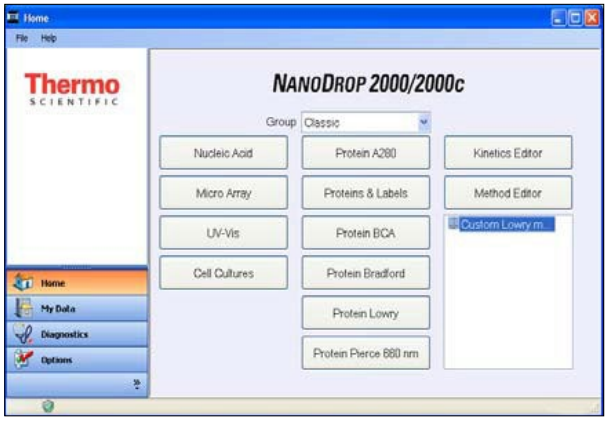
Step 7: Instrument Settings

Select the "Visible range" option for measurement range. It is essential that the wavelength range be wide enough to encompass the normalization wavelength and the entire analysis peak, including where the peak signal decreases to background levels.



Using the New Custom Method

Once saved, the method may be selected by clicking on the name of the method in the custom methods list. The default location for the method is in the "Classic" Group; however custom methods can be included in other groups if the user prefers.



Once the custom method has been initiated and measurement of the standard curve is complete, the absorbance values for each replicate of each standard along with the calculated mean absorbance value for each standard concentration will be displayed (Figure 2). In addition, the software enables the user to view the spectral output by selecting the "Data" tab (Figure 2). The final standard curve with the best fit line may be viewed by selecting the "Standard Curve(s)" tab (Figure 3).

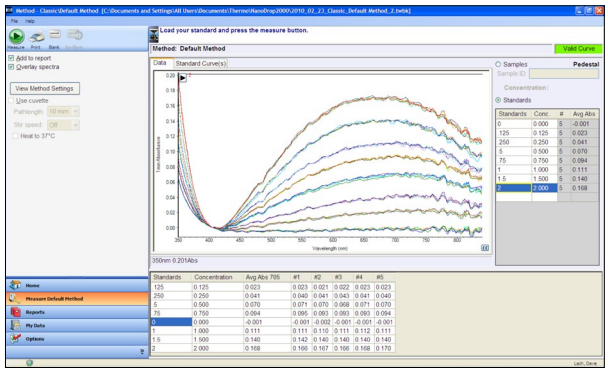


Figure 2: Standard curve data for a typical Lowry assay showing the absorbance values and spectra for each replicate.

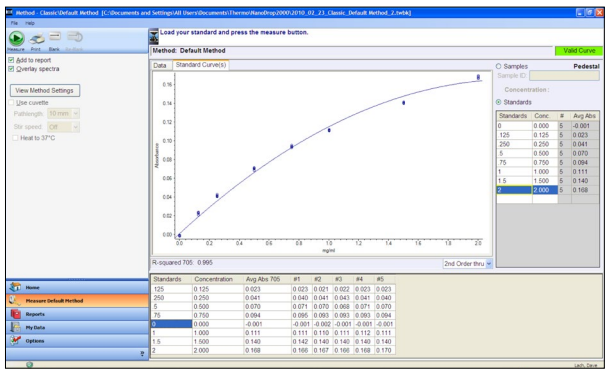


Figure 3: Standard curve for a typical Lowry assay showing the standard curve of absorbance of each standard after incubation with the Lowry reagent. Note that the relationship between the absorbance and the protein concentration is not linear.

Conclusion

The Method Editor is an easy step-by-step way to create a method when a new absorbance based assay is developed or experimental method is required. The standard curve based example presented here demonstrates the inherent versatility of the software. The NanoDrop pedestal technology allows the user to scale down assay volumes to save reagents, samples and costly disposables when creating a new assay or adapting a cuvette based assay to the NanoDrop 2000/2000c microvolume format. The ability to easily write custom methods is key in new method development; the ease of use of the NanoDrop 2000/2000c Method Editor makes it an ideal tool for this purpose.

Reference

1. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (November 1951). "Protein measurement with the Folin phenol reagent". *J. Biol. Chem.* 193 (1): 265–75.

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