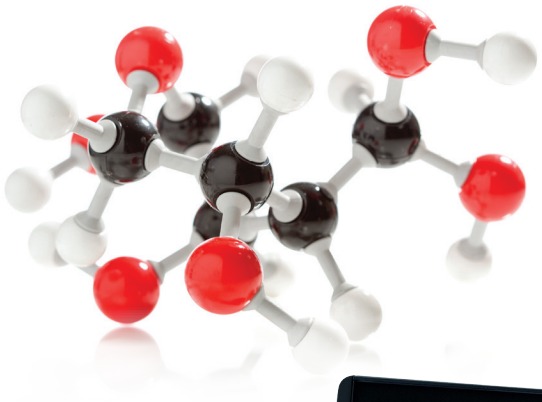
Quantifying glucose concentration using a hexokinase assay

Using the NanoDrop One Spectrophotometer

Abstract

Life scientists have used Thermo Scientific™ NanoDrop™ Microvolume UV-Vis Spectrophotometers for over a decade to accurately quantitate nucleic acid and protein concentrations in 1 or 2 µL of sample. Here we show how the NanoDrop One/One^{C} Spectrophotometer can be used to measure glucose concentration using the glucose hexokinase (Glucose HK) assay. This new custom method allows researchers to quantitate another common biomolecule, glucose, using minimal amount of their precious sample.



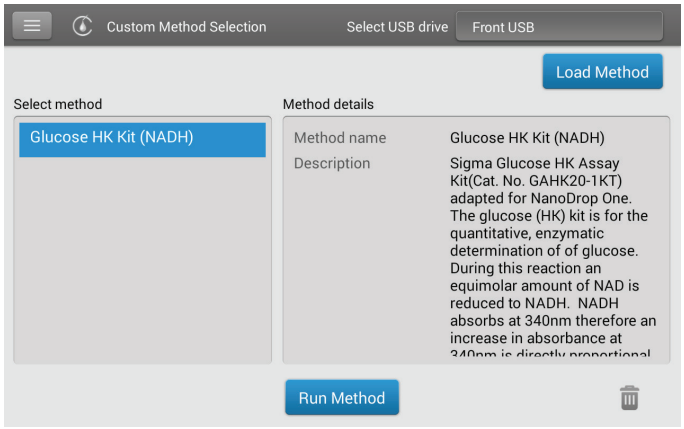


Figure 1. The Custom Method Selection screen is where users upload methods onto the instrument and run uploaded methods.

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Custom Method Download

1. Navigate to www.thermofisher.com/nanodrop
2. On the left, select “NanoDrop Software Download”
3. Choose the “NanoDrop One/One^{C}” tab
4. Select “Local Control Software Download Instructions”
5. Scroll to “How to add a NanoDrop One/One^{C} Custom Method file” and click on “Glucose HK Kit (NADH) Method”
6. Unzip the custom method file and copy the .method file to a USB device and then follow the online “Instructions for uploading a Custom Method to the instrument from a USB device”.

Introduction

Glucose is the primary energy source for a vast number of organisms. As such, quantitating glucose concentration can be a useful tool in various scientific endeavors including cancer research and plant biology. Many methods for quantitating glucose concentration have been developed [1]; however, accurate, direct quantitation of glucose with a spectrophotometer can be difficult. Glucose is typically found in samples containing nucleic acid and proteins. All three of these molecules absorb light in the UV region of the spectrum. With the current technology, UV-Vis spectrophotometers are unable to differentiate the absorbance of different molecules at the same wavelength, making it challenging to directly quantitate glucose in a sample.

This application note describes how the NanoDrop One/One^{C} Spectrophotometer can quantitate glucose utilizing the Sigma-Aldrich® Glucose (HK) Assay Kit (product number GAHK-20) [2]. This kit adds a layer of specificity to the measurement by reacting glucose with ATP and hexokinase to produce glucose-6-phosphate. The final product of this assay is NADH, which is generated when glucose-6-phosphate and NAD are reacted with glucose-6-phosphate dehydrogenase. Figure 2 shows how the stoichiometry of the reaction ensures the concentration of NADH produced is equivalent to the starting concentration of glucose. NADH absorbs light at 340 nm and the absorbance at this wavelength is used here to determine the glucose concentration of the sample. An example spectrum of NADH can be seen in Figure 3. This application note will discuss the assay, describe how to analyze the results, and present performance data for the reaction.

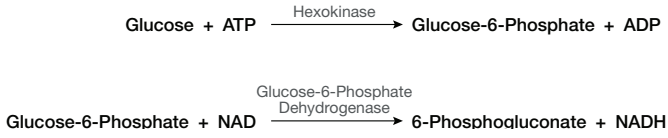

Figure 2. The equimolar production of NADH from glucose. The enzymatic activity of hexokinase and glucose-6-phosphate dehydrogenase produces 6-phosphogluconate and NADH from glucose and ATP.

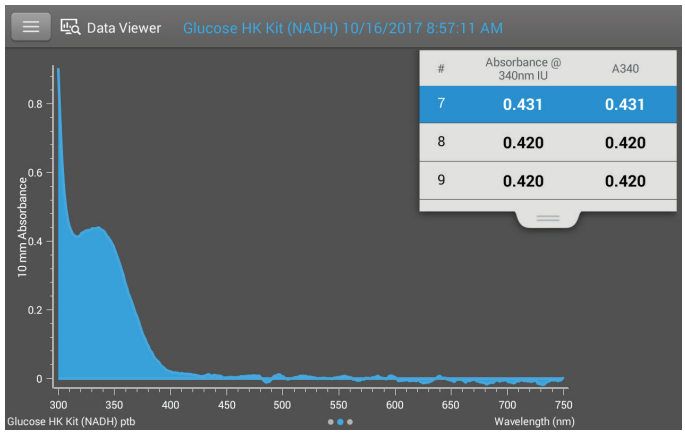

Figure 3. An example NADH spectrum. The concentration of NADH produced is equivalent to the starting concentration of glucose.

Measuring the glucose assay and analyzing data

The Sigma-Aldrich Glucose (HK) Assay comes with a 1 mg/mL glucose standard and the assay reagent. The assay can be performed with a sample-to-reagent ratio range of 1:5 to 1:100. This application note provides performance data for the 1:100 sample-to-reagent ratio reactions. The best sample-to-reagent ratio for the user is the one that is linear in the concentration range that includes their expected sample concentrations. Empirical studies can be performed with the provided glucose standard to optimize the ratio for the researcher’s needs.

The NanoDrop One/One^{C} glucose hexokinase custom method measures the absorbance at 340 nm and performs a baseline correction at 750 nm. Once the 340 nm absorbance is obtained, calculations need to be performed outside the NanoDrop One/One^{C} software to determine the glucose concentration. A Microsoft® Excel® spreadsheet is included with the custom method download that contains the equations needed. The researcher simply inputs the values for the total assay volume, dilution factor, sample volume, and 340 nm absorbance. The spreadsheet will calculate the glucose concentration for each sample.

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Glucose assay performance data

To assess the accuracy of Sigma-Aldrich Glucose (HK) Assay measurements on the NanoDrop One/One^{C} Spectrophotometer, the provided 1 mg/mL glucose standard was diluted down to 0.05 mg/mL. A sample-to-reagent ratio of 1:100 was used and three replicate measurements were made of each concentration. In our testing, the 1:100 sample-to-reagent ratio reactions were not linear below 0.25 mg/mL. Between 0.25 and 1 mg/mL the assay was linear with an R-squared value of 0.9911.

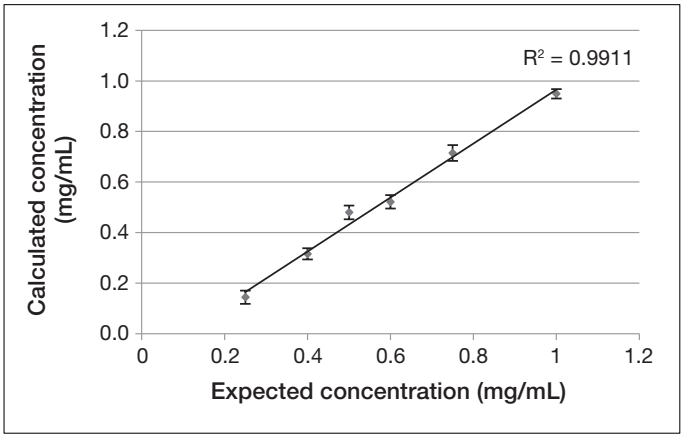

Figure 4. Glucose assay performance. Dilutions of glucose were prepared and measured using the Sigma-Aldrich Glucose (HK) Assay. The calculated results were plotted against the expected concentration. Error bars are standard deviation; n = 3.

Conclusion

This application note shows the NanoDrop One/One^{C} Spectrophotometer can be used to measure the glucose concentration of a sample using the hexokinase reaction. The data presented here verify the linear range of the 1:100 sample-to-reagent ratio reactions. Researchers can use the method and spreadsheet available for download to measure their samples and analyze their data.

This new method increases the range of uses for the NanoDrop One/One^{C} Microvolume Spectrophotometer.

Reference Literature

1. D. A. T. Southgate, Determination of food carbohydrates (Elsevier Applied Science; Elsevier Science Pub. [distributor], London; New York; New York, NY, USA, 1991).
2. Sigma-Aldrich, Glucose (HK) Assay Kit GAHK20 (available at https://www.sigmaaldrich.com/catalog/product/sigma/gahk20 accessed 30 Jan 2018).

Further Assistance and Technical Support

For further assistance, contact NanoDrop technical support at nanodrop@thermofisher.com or visit thermofisher.com/nanodrop.

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