Controls for Nucleic Acid Measurements

Introduction

This document provides guidance as to the correct use of the terms control and standard and the use of each with Thermo Scientific NanoDrop Spectrophotometers.

Standards vs. Controls

A "Standard" is generally accepted as a solution of a known concentration that is used to calibrate or certify that an instrument is working within acceptable, pre-defined guidelines.

A "Control" is a solution that produces an expected result within a specific range if the "system" is working as expected. The definition of system would include the instrument, protocols being used, techniques employed by the user and the solution utilized as the control.

Pedestal Pathlength Calibration Verification

Spectrophotometers measure light transmittance from which absorbance is calculated. When pedestal measurements are made using the NanoDrop™ Spectrophotometers, the software calculates the sample concentrations using Beer's law. This calculates concentration based upon absorbance, the appropriate molar extinction coefficient and pathlengths used. The calibration check will specifically assess the accuracy of the pathlengths used in these calculations. Wavelength calibrations using standard reference lines in the xenon spectrum are performed when the NanoDrop 2000/2000c software is opened. The software for the NanoDrop 1000 and NanoDrop 8000 Spectrophotometers performs wavelengths calibrations whenever an application module is opened.

CF-1 is a concentrated aqueous potassium dichromate ($K_2Cr_2O_7$) standard solution used to photometrically certify that the pathlengths utilized for pedestal measurements on the NanoDrop 2000/2000c are within calibration specifications. The solution is gravimetrically prepared using 0.673 g NIST SRM935a potassium dichromate and verified using an independently certified reference UV-Vis spectrophotometer (Agilent 8453). The calibration check software is accessed from the Diagnostics selection on the left pane of the operating software.

It is good practice to check the instrument's performance every six months with a fresh vial of CF-1. CF-1 is manufactured exclusively for use with NanoDrop Spectrophotometers and is available from Thermo Fisher Scientific and its distributors.

Nucleic Acid Controls

There are several nucleic acid products available that are appropriate for use as routine laboratory **control** solutions to monitor the reproducibility between replicates and monitor values obtained from day to day use. As discussed above, the controls are valid to use as long as the instrument is calibrated and the control product itself is within the expected concentration range stated in the manufacturer's specifications. The spectra generated from the control solutions may also be used as examples of what high quality purified nucleic acid spectra should look like.

For Technical Support contact us at 302-479-7707 or nanodrop@thermofisher.com.

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