

Cuvette Absorbance Verification Protocol

Technical Note 174

Introduction

The DS-11 Spectrophotometer Series includes three instruments with a cuvette-based UV-Vis measurement mode. These instruments include the DS-11+ Spectrophotometer, DS-11 FX+ Spectrophotometer / Fluorometer, and the DS-C Cuvette Spectrophotometer.

DeNovix cat# CUV-NA is an aqueous nicotinic acid reference solution. Although routine calibration is not necessary, the CUV-NA reagent may be used with the specified protocol to confirm that the instrument is performing within specifications.

Reagents

- **Always use a fresh vial for each verification check procedure.**
- The CUV-NA solution is supplied in a single use ampule that should be used immediately upon opening.
- Significant changes in concentration and possible verification check failures may occur if the vials are opened for longer than 1 hour prior to use.
- Only the CUV-NA standard from DeNovix and its authorized distributors should be used for the verification check.
 - No other sources of nicotinic acid are tested and validated for use with the DS-11+ and DS-C verification check procedure.
- The solution is light sensitive. Store unopened ampules in a dark, dry area at ambient temperature.
- CUV-NA solution is temperature sensitive. Do not hold the vial or cuvette in your hand for an extended period of time.

Protocol

1. Launch the **Formula Methods** app.
2. Tap **Create New** or select **New Method** using the **Expand** button (three vertical dots adjacent to method drop down list).
3. Define the method parameters as follows:
 - Method Name: **Cuvette IQOQ**
 - Analysis nm: **261**
 - Min nm: **220**
 - Max nm: **350**
 - Baseline nm: **340**
 - Tap the **Advanced** checkbox and select the **Savitzky-Golay** option
4. Follow the on-screen prompts regarding cleaning the microvolume sample surfaces and removing cuvettes from the holder and then tap **OK** to complete the method flash lamp optimization process.
5. Use the mode dropdown menu to select **Cuvette IQOQ (10mm)**.
6. Transfer ~3 mL of dH₂O into a quartz cuvette and insert it into the cuvette block. Use the etched light path arrow as a guide for proper cuvette orientation. Lower the arm and tap **Blank**.
7. Vigorously shake the nicotinic acid vial to thoroughly mix the solution.
 - Ensure all of the solution is in the bottom portion of the vial before opening the vial.
8. Transfer ~3 mL of the solution into a quartz cuvette and insert it into the cuvette block. Use the etched light path arrow as a guide for proper cuvette orientation. Lower the arm and tap **Measure**.
9. Take 5 replicate measurements.
10. Calculate the average measured absorbance value for the CUV-NA at 261 nm.



Results

Calculate the percent error using the following procedure:

1. Calculate the average measured absorbance value for the CUV-NA at 261 nm.
2. Calculate the % Error using the average of the replicates as the measured value in the following equation.

$$\% \text{ Error} = \left(\frac{\text{Measured} - \text{Target}}{\text{Target}} \right) \cdot 100$$

The absorbance specification for CUV-NA at 261 nm is +/- 3.0% error from the target value.

Troubleshooting

If results are not within +/- 3.0% error of expected absorbance, please see the list of possible causes below for troubleshooting advice.

- Cuvette was inserted in the improper orientation.
 - *Use the etched arrow to orient the transparent sides of the cuvette according to the indicated light path.*
- Cuvette was not UV-transparent.
 - *Use a quartz cuvette.*
- The solution has concentrated due to prolonged exposure.
 - *Use a fresh vial of solution.*
- An improper solution was used as a blank.
 - *Re-blank with a fresh sample of dH₂O, then remeasure the solution.*

Customer Support

If technical support is required, please contact DeNovix via email at info@denovix.com or call us at 302.442.6911.

- Please include the serial number of the instrument and provide your full contact information (email address and phone number) in the body of the email.
- If contacting us by phone, please have the replicate values handy to expedite the troubleshooting process.

Outside of the US, please contact your local distributor for assistance.

DeNovix Inc.
3411 Silverside Road
Wilmington, DE 19810 USA

Phone: +1.302.442.6911
Email: info@denovix.com
www.denovix.com

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