

# Reflection Linearity Standards Kit



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# Calibration of the ValPro™ Reflection Linearity Standards Kit

This document describes the polystyrene reflection linearity standard for near-infrared systems and the calibration process and report.

## General

The Thermo Scientific Reflection Linearity Standards Kit contains six reflection standards produced by Labsphere® that can be used to verify the performance of the Antaris™ Analyzer, specifically the integrating sphere module and the SabIR fiber optic probe. As a result of the calibration described in this document, five of the reflection standards are traceable to a photometric linearity standard set that was certified by the National Institute of Standards and Technology (NIST). The sixth sample, the 99% reflection standard, is used as a reference for the other sample measurements.

The standards are described below and are labeled with an identification number supplied by Labsphere. Each standard has a unique identifier that represents the nominal reflection. (The complete identification number of each reflection standard is written on the back of the standard. For example, if the identification number is SRS-80-010, the ID column is -80 and the nominal reflection is 80%.)

(-02) A nominal 2% reflection standard. This standard reflects approximately 2% of the radiant energy over the spectral range from 12,000  $\text{cm}^{-1}$  to 3,800  $\text{cm}^{-1}$  (833 nm to 2,632 nm).

(-10) A nominal 10% reflection standard. This standard reflects approximately 10% of the radiant energy over the spectral range from 12,000  $\text{cm}^{-1}$  to 3,800  $\text{cm}^{-1}$  (833 nm to 2,632 nm).

(-20) A nominal 20% reflection standard. This standard reflects approximately 20% of the radiant energy over the spectral range from 12,000  $\text{cm}^{-1}$  to 3,800  $\text{cm}^{-1}$  (833 nm to 2,632 nm).

(-40) A nominal 40% reflection standard. This standard reflects approximately 40% of the radiant energy over the spectral range from 12,000  $\text{cm}^{-1}$  to 3,800  $\text{cm}^{-1}$  (833 nm to 2,632 nm).

(-80) A nominal 80% reflection standard. This standard reflects approximately 80% of the radiant energy over the spectral range from 12,000  $\text{cm}^{-1}$  to 3,800  $\text{cm}^{-1}$  (833 nm to 2,632 nm).

## Standards for the measurement of the reflection standards

Thermo Fisher Scientific uses a set of neutral density filters that were measured and certified by NIST for traceability. These certified standards are used to calibrate the reflection linearity standards and demonstrate the photometric linearity of the Antaris analyzer. Although the entire spectrum is measured for each reflection standard, three specific wavelength values are used to demonstrate the linearity of the analyzer: 8,333  $\text{cm}^{-1}$  (1,200 nm), 6,250  $\text{cm}^{-1}$  (1,600 nm), and 5,000  $\text{cm}^{-1}$  (2,000 nm). Each  $\text{Log}(1/R)$  value is calculated by taking the average value measured in a 50  $\text{cm}^{-1}$  region centered at the wavelength of interest.

## Calibrating the reflection standards

The following is the procedure for measuring the reflection standards so that they are traceable. (It is technically correct to say the reflection standards are not traceable until the calibration is completed and the report is approved, but to improve the clarity, the reflection standards are referred to as traceable throughout these instructions.)

The procedure for measuring each reflectance standards kit includes the following steps:

- 1. Run the experiment on an Antaris analyzer that has passed the ValPro Instrument Qualification Test, Algorithm Qualification Test, and the Integrating Sphere Qualification Using Internal Standards test.
- 2. Insert the certified neutral density standards and measure all of them.
- 3. As prompted, insert a traceable Labsphere reflection standard into the sampling position on the Antaris analyzer. Before each measurement, the 99% reflection standard will be used to measure the background (reference) intensity.
- 4. Repeat step 3 for all reflection standards.

- 5. Repeat steps 2, 3, and 4 to take duplicate measurements of the certified standards and the traceable standards.
- 6. Report the results of the measurements in a report that is signed. The signed report demonstrates the traceability of the Labsphere reflection standards.

## **Uncertainty in the calibration measurements**

The calibration results for the five reflection standards are reported in  $\log(1/R)$  (equivalent to absorbance units), and values are given at three wavelengths for each reflection standard. The uncertainty of these values (computed by propagating the uncertainty from the NIST measurement) is expressed as one standard deviation in reflectance units and is 0.0023 for the measurements at  $8,333\text{ cm}^{-1}$  (1,200 nm) and  $6,250\text{ cm}^{-1}$  (1,600 nm). For measurements at  $5,000\text{ cm}^{-1}$  (2,000 nm), the uncertainty is 0.0050.

## **Expiration of the calibration of the reflection standards**

Every year that they are usable, the reflection standards must be recertified by the date shown on the Recertification Due sticker. The calibration of the reflection standards is accurate, within the measurement uncertainties specified, for two years from the date of issue. The reflection standards are stored under controlled conditions of registration and access by Thermo Fisher Scientific and are not released, even temporarily, from this controlled storage prior to issue.

## **Care of the reflection standards**

The handling procedures for Spectralon, provided by Labsphere, should be carefully followed. The reflection standards are delicate optical standards and must be treated with care. Contaminants such as skin oils can affect the reflectance, so Thermo Fisher Scientific and Labsphere recommend wearing clean gloves when handling the reflection standards. Spectralon can be cleaned if it is being used as a background, but in all other cases, Thermo Fisher Scientific does not recommend cleaning the certified standards because it may affect the calibration.

## **The calibration report**

The one-page calibration report for the traceable reflection standards kit is attached to this document. The details of the calibration follow the recommendations of the United States Pharmacopeia tests for qualification and verification (USP, General Chapter <1119> “Near-Infrared Spectrophotometry”).

The serial numbers of the analyzer used in the calibration and the certified standards (which are also used for the calibration and are traceable to NIST) are included in the report, along with the date of the action, the dated approval signature, and the issue date (which is the date the reflection standards kit was approved for shipment).

The report calibrates the photometric linearity reflection standards in the kit and makes them traceable to certified NIST standards. The report is traceable to the Labsphere report number, and both the report and the calibration file are archived at Thermo Fisher Scientific. At the end of the report is a certificate that confirms traceability to a NIST report.

## **Linearity calibration of the reflection standards kit**

When the reflection standard is measured, three values are reported: the  $\log(1/R)$  values defined at  $8,333\text{ cm}^{-1}$  (1,200 nm),  $6,250\text{ cm}^{-1}$  (1,600 nm), and  $5,000\text{ cm}^{-1}$  (2,000 nm). The background used is the 99% reflection standard. The first two sections comprise the calibration process, and the third verifies the results of the reflection standards kit.

There are four columns, including the “ID” column, which show the number of the individual reflection standards. The complete identification number of each reflection standard is written on the back of the standard. For example, if the identification number is SRS-80-010, the ID column is –80 and the nominal reflection is 80%. Values are reported in the columns following the ID column and are calculated for each of the five reflection standards for  $\text{Log}(1/R)$  at  $8,333\text{ cm}^{-1}$  (1,200 nm),  $6,250\text{ cm}^{-1}$  (1,600 nm), and  $5,000\text{ cm}^{-1}$  (2,000 nm).

## **Measurement of the certified standards**

The results of the measurements of the certified standards that are used to calibrate the reflection standard are shown in the “Actual” and “Meas” columns. The Actual column shows the intensity of the signal at the reported wavelength as reported by the NIST measurement. The Meas column shows the intensities of the signals measured by the two experiments on the certified standards in the serialized analyzer. Values are reported for the five neutral density filters, and a regression is performed between the actual values and the measured values at each of the three spectral locations. The slope is reported and must fall between 0.95 and 1.05 inclusive. The intercept is also reported and must fall between  $-0.05$  and  $+0.05$ , inclusive. The regression equations for the three columns are used to calibrate the traceable standards.

## **Measurement of the traceable reflection standards**

The section of the report titled “Measurement of the Traceable Reflection Standards” shows the results of the measurement of the traceable standards compared to the measurements made on the certified reflection standards. The “Meas” column shows the intensities of the signals measured by the two experiments on the traceable reflection standards. The “Interp” column shows the intensities of the measured signals when corrected by the regression equation generated in the “Measurement of the Certified Standards” section of the report. These numbers are calibrated and traceable to the “Actual” values as reported for the certified reflection standards.

## **Traceable reflection standard results summary**

This section shows the  $\log(1/R)$  values used to describe (calibrate) the traceable reflection standard set. Each measurement is the average of the two associated interpolated values taken from the table in the “Measurement of the Traceable Reflection Standards” section. These values are taken as the true measurement values for the traceable Labsphere reflection standard set.

## **Confirming that the reported values match the values in the Antaris analyzer**

To verify that the information from this report has been installed on your Antaris analyzer, choose Instrument Status from the Maintenance menu in the RESULT Operation software. This opens the Instrument Status Report Options dialog box. Select Show External Validation Information under Report Options, and then click OK.

A report of the analyzer status will appear in the window. The contents of this report depend on the selections you make in the Instrument Status Report Options dialog box. If the proper information is available on the Antaris analyzer, there will be a section beneath the heading “External reflectance standards for linearity” that gives the serial number information for the standards kit is shown. The last column of the table beneath the heading contains values that should match those in the “Traceable Reflection Standards Results Summary” section of the attached reports. If these values do not match, contact Thermo Fisher Scientific.

## **Agreement with the report issued by Labsphere for the reflection standards**

The values reported in the Thermo Fisher Scientific report may differ from the reported Labsphere values. As discussed in the United States Pharmacopeia (USP, General Chapter <1119> “Near-Infrared Spectrophotometry”), photometric qualification of linearity should be based on a set of reflection standards. According to the USP:

*Spectra obtained from reflectance standards are subject to variability due to the difference between the experimental conditions under which they were factory-calibrated and those under which they were subsequently put to use. Hence, the percent reflectance standards supplied with a set of calibration standards may not be useful in the attempt to establish an “absolute” calibration for a given instrument. ... Subsequent measurements on the identical set of standards give information on the long-term stability.*

The experimental conditions under which the standards were factory-calibrated at Labsphere and those under which they were subsequently put to use at Thermo Fisher Scientific and at the customer site differ. The Labsphere values use a different type of integrating

sphere and spectrophotometer. Consequently, the values that Labsphere has measured and supplied can differ from the values measured on an Antaris analyzer, as acknowledged in the above quote from the USP.

The experimental difference between the Thermo Fisher Scientific factory-calibration and your customer site are not significant because Antaris analyzers have been designed and manufactured to give similar performance. Therefore the Thermo Fisher Scientific-supplied factory-calibration will demonstrate the USP requirements for photometric linearity required on your analyzer and can be used to demonstrate the long-term stability of the photometric linearity.





