

# UV/VIS Spectrophotometers

## Calibration Guide



## Calibration set EMC-SET-100

Glass filter set EMC-SET-100 contains:

### Wavelength Accuracy:

H1 Holmiumoxide filter

### Photometric Accuracy:

N2 Neutral density glass filter 0.25 Abs

N3 Neutral density glass filter 0.5 Abs

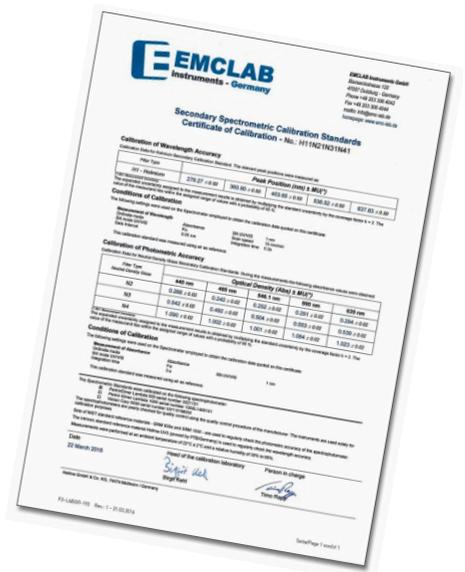
N4 Neutral density glass filter 1.0 Abs

N0 Blank filter

Calibration Certificate

Microfiber cloth

Calibration label (2 pcs.)



# General Information

## General Usage Guidelines for Glass Filters

Glass filters are made of glass doped with metal ions/rare earth metals, which are assembled stress-free in plastic frames. They are designed to fit into all spectrophotometers equipped with a holder for standard cuvettes with a 10-mm optical path length. To ensure that filters can be easily identified, each filter frame is engraved with the filter type and serial number. Details of the absorbance and peak position values measured for each filter can be found on the respective Calibration Certificate. Please ensure that you do not touch the glass surfaces of the filter. Dirt, dust, and damage can significantly impair the accuracy of measurement results. The plastic frames should not come into contact with acids or alkalis. When inserting the filter into the spectrophotometer, no pull, pressure or lever should act on the holder.

## Storage

After use, we strongly recommend storing the filters at room temperature, in their packaging, and in a dry, dust free area.

## Cleaning

Dirt often accumulates on optical surfaces as a result of regular use. This is best removed using a lint-free cloth and alcohol (Attention: Never clean the plastic frame with alkaline concentrate solution!)

## Influence of temperature and humidity on measurements

Temperature has only an exceedingly small influence on the certified measurement values, and temperatures between 20°C and 24°C fall within the measurement uncertainty stated on the calibration certificate. Measurements should therefore be performed within this range to avoid any potential temperature influence on the results to a minimum. Take care that the relative humidity is between 30% to 65%, especially when measuring within UV range.

## Other factors that may influence the measurements

Dirt (e.g. fingerprints) and dust on, or damage (scratches, corrosion) to, polished optical surfaces can significantly impair the accuracy of measurement results. Always store the filters in their original packaging and protect the optical windows from contamination. Only handle the filters by their frames.

## Steps to take before performing calibration

- Switch on and preheat the instrument for at least 20 minutes
- Only use a stable cell holder for 10 mm standard cells. Make sure that the holder is secure and stable in the sample compartment.
- Carry out a wavelength reset and dark current.
- Carry out a baseline correction with an empty sample compartment.
- Make sure all filters are always clean before using. Otherwise, please use the provided microfiber cloth for purifying.
- Check that the filter is correctly positioned in the light path by first placing the blank filter N0 in the cell holder. The N0 marking must be visible from above. Ensure that all filter frames are always positioned in the same way, i.e. with serial numbers facing the detector.
- Choose the slit width the filters are calibrated at (4 nm for EMC-SET-100) or the slit width that is closest. If you cannot adjust the slit width of the instrument, note that slight deviations to the values recorded in the calibration may appear. (recommended rough guide: double uncertainty if slit width differs from certified slit width)
- Carry out measurement of the filters just as carefully as you would carry out a sample measurement in a closed sample compartment (opening the sample compartment will cause incorrect results).

## Neutral Density Glass Filter for checking Photometric Accuracy

To measure photometric accuracy (absorbance), the filter reduces the light beam from the spectrophotometer. An absorbance value (Abs) can be deduced from the light extinction caused by the filter. The present Neutral Density Glass Filters are made from special selected filter materials. They show a relatively constant transmittance within the wavelength range of 405 nm to 800 nm, therefore they are an excellent choice to check photometric accuracy in the visible wavelength range (> 405 nm).

<b>Type</b>	N2, N3, N4, Neutral Density Glass Filter
<b>Application</b>	Testing the photometric accuracy in the VIS range
<b>Content</b>	N2: Neutral Density Glass Filter (0,25 Abs), N3: Neutral Density Glass Filter (0,5 Abs), N4: Neutral Density Glass Filter (1,0 Abs),
<b>Standard Certification</b>	Wavelengths: 440, 465, 546.1, 590, 635 nm Slit width: 1 nm

# Preparation for calibration

## Holmium Glass Filter for checking Wavelength Accuracy

To measure wavelength accuracy, the filter absorbs the light beam of the spectrophotometer to a greater extent at certain wavelengths creating absorbance peaks. The Holmium Glass Filter is an excellent choice for checking the wavelength scale of a spectrophotometer since it has a range of narrow, well defined peaks in the UV and visible range.

<b>Typ</b>	H1, Holmiumoxide Glass Filter
<b>Application</b>	Testing the wavelength accuracy in the UV and VIS range
<b>Standard Certification</b>	Wavelength accuracy at 279 nm to 638 nm Slit width: 1 nm

**EMCLAB Instruments GmbH**



<p><b>Kalibriergegenstand</b> Spectrophotometer</p> <p><b>Kalibrierverfahren</b> Messung der photometrischen Genauigkeit</p> <p><b>Kalibrierungsmethode</b> Measurement of photometric accuracy</p> <p><b>Messtechnische Bedingungen</b> Die in diesem Kalibrierzertifikat angegebenen Werte wurden mit dem verwendeten Spektrophotometer und den nachstehenden Einstellungen ermittelt:</p> <p><b>Modus der Ordinatenskala</b> Optische Dichte (Abs.)</p> <p><b>Spaltbreite</b> 4 nm</p> <p><b>Spaltmodus</b> F1</p> <p><b>Conditions of Calibration</b> The following settings were used on the spectrophotometer employed to obtain the data quoted on this calibration certificate:</p> <p><b>Ordinate mode</b> Optical density (Abs.)</p> <p><b>Slit</b> 4 nm</p> <p><b>Slit mode</b> F1</p>	<p>Für die Kalibrierung dieses Kalibriergegenstandes wurden nachstehende Filter, kalibriert vom DAkkS Kalibrierlabor D-K-18752-01-00, eingesetzt:</p> <p>Holmiumoxid-Glasfilter F1, Serien-Nr. 5440, Kalibrierschein 18282</p> <p>Neutralfilter F2, F3, F4, Serien-Nr. 5440, Kalibrierschein Nr. 18283</p> <p>Flüssigfilter UV60, Serien-Nr. 0940, Kalibrierschein Nr. 18288</p> <p>Flüssigfilter UV600, Serien-Nr. 0905, Kalibrierschein Nr. 18288</p> <p>Referenzfilter UV14, Serien-Nr. 1196, Kalibrierschein Nr. 18288</p> <p>Die Filter werden regelmäßig auf die Einhaltung ihrer Spezifikationen überprüft. Datum der letzten Kalibrierung: 2013-12-12</p> <p>This calibration object was calibrated with the following filters calibrated from DAkkS calibration laboratory D-K-18752-01-00:</p> <p>Holmium Oxide Glass Filter F1, serial no. 5440, calibration certificate 18282</p> <p>Neutral Density Glass Filters F2, F3, F4, serial no. 5440, calibration certificate 18283</p> <p>Liquid Filter UV60, serial no. 0940, calibration certificate 18288</p> <p>Liquid Filter UV600, serial no. 0905, calibration certificate 18288</p> <p>Reference Filter UV14, serial no. 1196, calibration certificate 18288</p> <p>The filters are regularly checked for the compliance with their specifications. Most recently calibration date: 2013-12-12</p>
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<p><b>Umgebungsbedingungen</b> 22 °C ± 2 °C, relative Luftfeuchtigkeit 30 % bis 65 %</p> <p><b>Environmental conditions</b> 22 °C ± 2 °C, relative humidity of 30 % to 65 %</p> <p><b>Messergebnisse</b> Während der Messung wurden die folgenden Werte ermittelt:</p> <p><b>Measurement results</b> During the measurements, the following data were obtained:</p>	
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Filter	Serien-Nr. / serial no.	235 nm	257 nm	313 nm	350 nm	430 nm
UV60 (K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> 60 mg/l)	8848 Lösung Nominal value	0.7502 ± 0.0003	0.8691 ± 0.0003	0.2041 ± 0.0003	0.6450 ± 0.0003	-
	Gemessener Wert Measured value	-	-	-	-	-
	9585 Lösung Nominal value	-	-	-	-	0.9530 ± 0.0005
UV600 (K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> 600 mg/l)	8848 Lösung Nominal value	-	-	-	-	-
	Gemessener Wert Measured value	-	-	-	-	-
	9585 Lösung Nominal value	-	-	-	-	-

Filter	Serien-Nr. / serial no.	440 nm	465 nm	546.1 nm	590 nm	635 nm
F2 (0,25 Abs.)	8549 Lösung Nominal value	0.2628 ± 0.0024	0.2370 ± 0.0024	0.2468 ± 0.0024	0.2870 ± 0.0024	0.2910 ± 0.0024
	Gemessener Wert Measured value	-	-	-	-	-
	8548 Lösung Nominal value	0.5353 ± 0.0021	0.4857 ± 0.0021	0.4975 ± 0.0021	0.5477 ± 0.0021	0.5355 ± 0.0021
F3 (0,5 Abs.)	8549 Lösung Nominal value	-	-	-	-	-
	Gemessener Wert Measured value	-	-	-	-	-
	8548 Lösung Nominal value	1.0745 ± 0.0045	0.9986 ± 0.0045	1.0038 ± 0.0045	1.0726 ± 0.0045	1.0397 ± 0.0045
F4 (1 Abs.)	8549 Lösung Nominal value	-	-	-	-	-
	Gemessener Wert Measured value	-	-	-	-	-
	8548 Lösung Nominal value	-	-	-	-	-

Photometrische Genauigkeit  
Photometric Accuracy

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Fig. 1 Calibration Certificate (sample) - Photometric Accuracy

## 1. Photometric Accuracy Checking

Filters: 0.25 Abs, 0.5 Abs, 1.0 Abs neutral density glass filters N2, N3, N4  
Test mode: Photometric  
Reference setting: Testing wavelengths 440, 465, 546.1, 590, 635 nm  
Display mode: A

1. First of all, follow the steps to take before performing calibration (page 4)
2. Set the display mode to absorbance (Abs).
3. Run the wavelength selection program on your spectrophotometer, observing the guidelines in the user manual. Select the wavelengths provided on the Calibration Certificate (see above reference setting).
4. Insert blank filter N0 as reference and carry out blank (100%T/0Abs).
5. Insert the neutral density filters N2, N3, N4. Ensure that the filters are inserted into the holder as far as possible. The N2, N3, N4 marking must be visible from above (serial number facing the detector).
6. Start the program for measuring the absorbance values at the aforementioned wavelengths stated on the calibration certificate. Record the values. Take several measurements (we recommend at least 3) and average the measured values to avoid errors.
7. Compare the measured values with the standard values in the calibration certificate.
8. Repeat the steps 1 to 7 for every given wavelength in the calibration certificate.

If you are using the PC software PROFESSIONAL, Easy UV or ANALYST, you can carry out the calibration and save results automatically by executing the "Multi Wavelength Scan" application.

# Testing and calibration

## 2. Wavelength Accuracy Checking

Filters: Holmiumoxide filter H1 (Fig. 2)  
Test mode: Spectrum Scan  
Reference setting: Peak wavelengths\* see calibration certificate  
Scanning interval: 0.5 nm  
Display mode: A



Fig. 2

1. First of all, follow the **steps to take before performing calibration**.
2. Set the display mode to absorbance (Abs).
3. Run the scan program on your spectrophotometer, observing the guidelines in the user manual. Select a scanning range that covers all the peaks listed on the Calibration Certificate (see above reference setting). If you cannot set a range, please follow the steps below for manual measurement.
4. Adjust your spectrophotometer to the measurement parameters that appear on the calibration certificate. Select a slow scanning speed and a small data interval.
5. Measurements are taken using an air blank which means reference measurement is taken using the blank filter N0.
6. Insert the Holmiumoxide filter H1. Ensure that the filter is inserted into the holder as far as it will go. The H1 marking must be visible from above (serial number facing the detector).
7. Start the measurement.
8. Take several measurements (we recommend at least 3) and average the measured values to avoid errors.
9. Compare the positions of the peaks to the wavelengths stated on the calibration certificate.

**If the scan program is not applicable you can do it manually:  
e.g. peak 360,90:**

1. Set the wavelength to 355 nm, insert blank filter N0 as reference and carry out blank (100%T/0Abs).
2. Insert Holmiumoxide filter H1. Ensure that the filter is inserted into the holder as far as it will go. The H1 marking must be visible from above (serial number facing the detector). Record the value.
3. Set the wavelength to the next higher value (+0.5 nm), repeat step 1 and 2 get the value during 355 nm to 366 nm.
4. Compare the measurement values with the certified ones.
5. Repeat steps 1 to 4 for every given peak position from the calibration certificate.

- Option 1: Wavelength scan with the On-board software (not applicable for all instruments)
- Option 2: Wavelength scan with PC software PROFESSIONAL, Easy UV or ANALYST

\*Wavelength 279 nm is within the UV range and not working in the VIS range

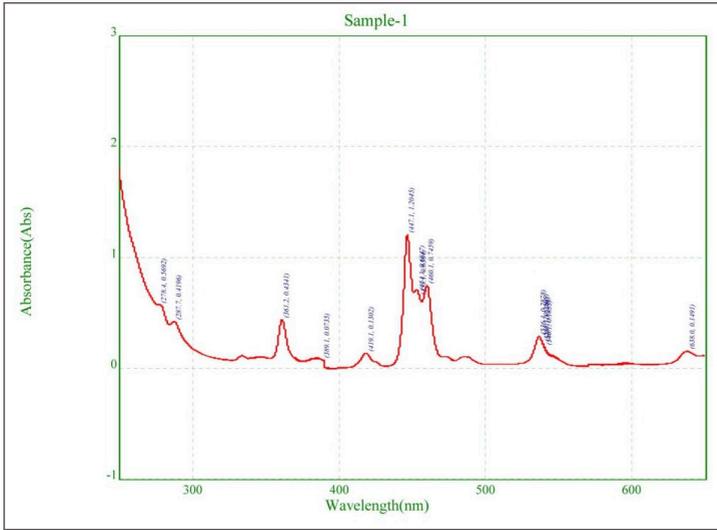


Fig. 3 Wavelength scan with Holmiumoxide filter H1 (ANALYST PC Software)

### 3. Stray Light Checking (filter optionally available\*\*)

Only for spectrophotometers that can measure in the UV range!

Filters: UV1, UV12 (reference filter) (Fig. 4)  
 Test mode: Spectrum Scan  
 Setting: 250 nm to 200 nm  
 Display mode: A

1. First of all, follow the **steps to take before performing calibration**.
2. Set the display mode to absorbance (Abs).
3. Run the scan program on your spectrophotometer, observing the guidelines in the user manual. Select the scanning range according to the setting above (250 nm to 200 nm).
4. Select a slow scanning speed and a small data interval.

# Testing and calibration

- Measurements are taken using a pure water blank which means reference measurement is taken using the filter UV12. Ensure that the filter is inserted into the holder as far as possible. Do a blank scan to set the instrument to zero.
- Insert the potassium chloride filter UV1. Ensure that the filter is inserted into the holder as far as it will go.
- Start the measurement.
- Take several measurements (we recommend at least 3) to avoid errors.
- Compare the curve shape to the characteristic curve (Fig. 5).

\*\*Optionally available DAkkS and NIST certified reference filters:

UV1 (Potassium chloride in pure water) + UV12 (pure water (H<sub>2</sub>O) reference filter)



Fig. 4 UV1 (KCl) UV12 (H<sub>2</sub>O)

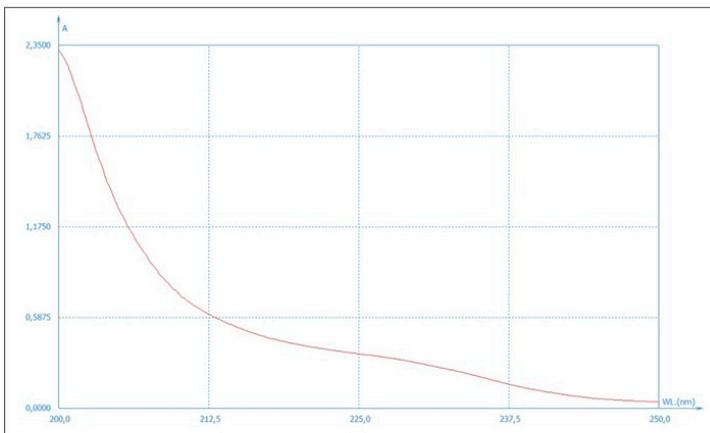


Fig. 5 Characteristic Curve Shape

## 4. Recertification

As it is the case for all measuring devices, the reference materials used to verify spectrophotometers must also be checked and recertified at regular intervals (see for example ISO 9001:2015 "Control of Monitoring and Measuring Equipment"). This allows you to ensure that you consistently fulfill your in-house quality requirements and guarantees high levels of accuracy and reliability in your measurements

The length of intervals between the recertification of reference materials depends on how frequently materials are used, the wear associated with this, accuracy requirements, as well as the company's internal auditing requirements. Therefore, only users themselves can determine these.

# Testing and calibration

## 5. Works Calibration Form

Together with the EMC-SET-100, EMCLAB Instruments provides a blank calibration form to enable the user to issue a works calibration certificate. To duplicate the calibration form or to issue a calibration certificate with printed instead of handwritten values, you can contact us to get the form as a file by Email.

Calibration object: Spectrophotometer  
 Photometric Accuracy of the object:  $\pm$  \_\_\_\_\_ % T or  $\pm$  \_\_\_\_\_ A@1A

**Calibration method**  
 Measurement of Absorbance (N2 – N4)

**Conditions of Calibration**  
 The following settings were used on the Spectrophotometer employed to obtain the calibration data quoted on this certificate:

Ordinate mode:	Absorbance
Slit UV/VIS:	1 nm
Slit mode UV/VIS:	Fix
Integration time:	3 s

**Measurement of Wavelength (H1)**

**Conditions of Calibration**  
 The following settings were used on the Spectrophotometer employed to obtain the calibration data quoted on this certificate:

Ordinate mode:	Absorbance
Slit UV/VIS:	1 nm
Slit mode UV/VIS:	Fix
Integration time:	0.2 s
Scan speed:	15 nm/min
Data interval:	0.05 nm

This calibration object was calibrated with the following filters from the Secondary Spectrometric Calibration Standards Certificate of Calibration No. H12N2N3N4C:  
 Neutral Density Glass Filter N2  
 Neutral Density Glass Filter N3  
 Neutral Density Glass Filter N4  
 Holmium Oxide Glass Filter H1

This calibration standard was measured using air as reference.  
 The Spectrometric Standards were calibrated on the following Spectrophotometer:  
 Varian Cary 5000 serial number UV1101M202.  
 The Spectrophotometer is yearly checked for quality control using the quality control procedure of the manufacturer. The instrument is used solely for calibration purposes.  
 Sets of NIST standard reference materials – SRM 930e and SRM 1930 – are used to regularly check the photometric accuracy of the spectrophotometer.  
 The intrinsic standard reference material Helma UV5 (proved by PTB/Germany) is used to regularly check the wavelength accuracy. Measurements were performed at an ambient temperature of 22°C  $\pm$  2°C and a relative humidity of 30% to 85%.

During the measurements the following absorbance values were obtained:

Filter Type Neutral Density Glass	Photometric Accuracy   Optical Density (Abs) $\pm$ MU(*)				
	440 nm	465 nm	546.1 nm	590 nm	635 nm
N2 (0.25 Abs)	0.266 $\pm$ 0.02	0.241 $\pm$ 0.02	0.252 $\pm$ 0.02	0.291 $\pm$ 0.02	0.294 $\pm$ 0.02
N3 (0.5 Abs)	0.541 $\pm$ 0.02	0.491 $\pm$ 0.02	0.503 $\pm$ 0.02	0.552 $\pm$ 0.02	0.538 $\pm$ 0.02
N4 (1 Abs)	1.092 $\pm$ 0.02	1.005 $\pm$ 0.02	1.003 $\pm$ 0.02	1.067 $\pm$ 0.02	1.026 $\pm$ 0.02

(\*) MU: Measurement Uncertainty

Filter Type Holmium	Wavelength Accuracy   Peak Position (nm) $\pm$ MU(*)				
	279.32 $\pm$ 0.50	360.85 $\pm$ 0.50	453.58 $\pm$ 0.50	536.42 $\pm$ 0.50	637.67 $\pm$ 0.50
H1					

(\*) MU: Measurement Uncertainty

Fig. 6 Blank Calibration Form



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