Certified Reference Materials for UV/Vis Spectroscopy

PRODUCT CATALOG WITH USAGE GUIDELINES

GLASS FILTERS
LIQUID FILTERS
REFERENCE PLATES
YOUR PROCESS. OUR SOLUTIONS.

As a leading supplier of high-precision, optical solutions that are ‘Made in Germany’ from glass, quartz glass and synthetic crystals, Hellma has been a by-word for outstanding quality for over 95 years. A key supplier, the company is an integral part of its clients’ value chains. Reliability, trust and continuity are inherent in Hellma’s work, and the company believes it has both a duty and responsibility to ensure these principles are upheld. Clients from more than 40 countries worldwide put their confidence in Hellma’s exceptional level of performance and problem-solving skills to meet and exceed regulatory requirements and to make their products safer.

Certified precision – reliable results

NIST, PTB
Traceable

DAkkS
accredited calibration laboratory

ACCREDITED
ACCORDING TO
DIN EN ISO / IEC 17025

30 YEAR
GUARANTEE
with regular recertification

COMPLIANT
with the most important Pharmacopeia

Benefit from more than 95 years of experience.
Where you see this icon you will find useful information to make your processes safer and more effective.

Hellma® Analytics
Optical components and assemblies used in devices and systems in analytical technology.

www.hellma-analytics.com

Hellma® Materials
High-quality synthetic crystals for use in the fields of microlithography, optics, laser technology and radiation detection.

www.hellma-materials.com

Hellma® Optics
Premium-quality precision optics for use in laser technology as well as all areas of photonics and the optical industry.

www.hellma-optics.com
# INTRODUCTION

1. Hellma Analytics calibration laboratory
2. Certified test equipment
3. Glass filter applications
4. Liquid filter applications
5. DAkkS calibration certificate
6. 30-year manufacturer’s warranty

# GLASS FILTERS

2.1 Checking wavelength accuracy
2.1.1 Holmium glass filter
2.1.2 Didymium glass filter
2.2 Checking the photometric accuracy
2.2.1 Didymium glass filter F7A
2.2.2 Neutral density glass filter
2.3 Glass filter sets
2.4 General usage guidelines for glass filters
2.5 Calibration with glass filters
2.5.1 Preparations
2.5.2 Steps for checking wavelength accuracy with holmium glass or didymium glass filter
2.5.3 Steps for checking photometric accuracy with a Neutral density glass filter or a didymium glass filter (F7A)
2.5.4 Calibration with glass filters – interpreting measurement results

# LIQUID FILTERS

3.1 Checking wavelength accuracy
3.1.1 Holmium liquid filter
3.1.2 Didymium liquid filter
3.1.3 HoDi liquid filter
3.1.4 Rare Earth liquid filter
3.2 Checking the photometric accuracy
3.2.1 Potassium dichromate liquid filter for checking the photometric accuracy in accordance to Ph.Eur and USP <857>
3.2.2 Niacin liquid filter
3.3 Checking for stray light

# REFERENCE PLATES

4.1 Checking the photometric accuracy
4.2 Checking photometric and wavelength accuracy
4.3 General usage guidelines for reference plates
4.4 Calibration with reference plates
4.4.1 Preparations
4.4.2 Steps for checking photometric accuracy with reference plates
4.4.3 Steps for checking wavelength accuracy with reference plates
4.5 Documented process reliability: With control charts for certified reference materials

# Recertification

5. Returning your reference materials for recertification

# FAQ

6.

# Glossary

7.

# Literature references

8.

# Product Overview

9.
Dear Readers,

Although checking measuring equipment to ensure that results are accurate has long been common practice for analytical balances, it still tends to take something of a backseat where spectrophotometers are concerned. Spectrophotometers are important instruments that play a major role in health care, the life sciences, environmental analysis and processes such as production control and ensuring product quality. Over the last two years, many laboratories have become considerably more aware of the need to check their spectrophotometers, making it all the more important to know that these precision tools are also subject to mandatory checks under DIN EN ISO 9001. The standard clearly stipulates that measuring equipment be calibrated or verified, either at regular intervals or before use, using measurement standards that can be traced back to international or national standards. For an overview of the measurement standards for UV-Vis spectrophotometers, please refer to our reference materials in this brochure. An increasing number of laboratories are turning to this easy method for ensuring high standards of work – not only to satisfy requirements in time for their next audit, but also to be safe in the knowledge that they are taking accurate measurements and thus basing their actions and responses on correct results. We are delighted that our products are helping to achieve this. Details of our product range, as well as usage guidelines, helpful tips, and recommendations, are all included in this Handbook. WE VERY MUCH HOPE THAT YOU ENJOY READING IT.

1.1 Hellma Analytics calibration laboratory: accredited to DIN EN ISO 17025

Our lab is a DAkkS calibration laboratory and is accredited to DIN EN ISO 17025, a comprehensive quality management system that acts as a seamless continuation of other systems such as ISO 9001. By achieving this accreditation, we have demonstrated proof of expertise in the calibration activities that we perform and are authorized to issue internationally recognized DAkkS calibration certificates. The accreditation is the key to high quality measurements, international comparability, and trust in both the work of the calibration laboratory and the transparency of results.

With the certified UV/Vis reference materials from Hellma Analytics, we create the basis for reliable measurements for our customers.
1. INTRODUCTION

1.2 Certified test equipment

Quality assurance and quality control regulations, such as ISO 9001, GLP, GMP, and Pharmacopeias, require companies to verify the consistently excellent performance of any spectrophotometer in use. The two most important factors for obtaining precise spectrophotometer data are the photometric accuracy (absorbance accuracy) and wavelength accuracy of the spectrophotometer, which should be tested on a regular basis.

In the Hellma Analytics calibration laboratory, which is accredited to DIN EN ISO 17025, we manufacture certified reference materials based on the regulatory codes issued by NIST (National Institute of Standards and Technology), ASTM (American Society for Testing and Materials) and Pharmacopeias (Ph. Eur, USP). All certified measurement results can be traced back to NIST (photometric accuracy) or to PTB (Physikalisch-Technische Bundesanstalt) (wavelength accuracy) standard reference materials. (Photometric accuracy: NIST SRM® 930e, NIST SRM® 1930. Hellma 6466300; wavelength accuracy: Hellma 667005)

Choose between glass filters and liquid filters for your reference materials:

1.3 Glass filter applications

666 at the beginning of the article number identifies our glass filters. Glass filters are certified reference materials made of glass manufactured specifically for calibration. They are, above all, extremely robust. All glass filters certified by Hellma Analytics are traceable to NIST primary standards. Certified glass filters are suitable for checking the following parameters of your spectrophotometer:

- Wavelength accuracy
- Photometric accuracy (absorbance)

1.4 Liquid filter applications

667 at the beginning of the article number identifies our liquid filters. Liquid filters are certified liquid reference materials that are manufactured in compliance with Pharmacopeias and/or NIST standards and filled into quartz glass cuvettes under controlled conditions. The cuvettes are then permanently sealed to become airtight. Liquid filters have the distinct advantage of equating to real measuring conditions. Certified Hellma Analytics liquid filters are suitable for checking the following parameters of your spectrophotometer:

- Wavelength accuracy
- Photometric accuracy (absorbance)
- Stray light levels
- Spectral resolution

You should regularly check your UV/Vis spectrophotometer for all of these parameters, especially photometric and wavelength accuracy, while observing the relevant requirements in your device handbook. Thanks to their ease of use and long service life, certified Hellma Analytics reference materials provide an excellent aid for all routine checks.

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>CHECKING OF</th>
<th>RANGE</th>
<th>PH.EUR 8.0</th>
<th>USP «857»</th>
<th>ASTM</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLASS FILTERS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holmium glass</td>
<td>Wavelength accuracy</td>
<td>UV/Vis</td>
<td></td>
<td></td>
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<tr>
<td>Didymium glass</td>
<td>Wavelength accuracy</td>
<td>UV/Vis</td>
<td></td>
<td></td>
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<tr>
<td>Neutral density glass</td>
<td>Photometric accuracy</td>
<td>Vis</td>
<td></td>
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<tr>
<td>LIQUID FILTERS</td>
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<tr>
<td>Holmium (solution)</td>
<td>Wavelength accuracy</td>
<td>UV/Vis</td>
<td></td>
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<tr>
<td>Didymium (solution)</td>
<td>Wavelength accuracy</td>
<td>UV/Vis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Potassium dichromate (solution)</td>
<td>Photometric accuracy</td>
<td>UV/Vis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Toluene in hexane (solution)</td>
<td>Spectral resolution</td>
<td>UV</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Potassium chloride (solution)</td>
<td>Stray light</td>
<td>UV</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sodium iodide (solution)</td>
<td>Stray light</td>
<td>UV</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sodium nitrite (solution)</td>
<td>Stray light</td>
<td>UV</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Acetone</td>
<td>Stray light</td>
<td>UV</td>
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</tbody>
</table>
After careful production, reference materials are certified in the Hellma Analytics calibration laboratory (accredited to DIN EN ISO 17025) using a high-performance UV-Vis/NIR spectrophotometer. Reference materials are only considered to be certified if they have been issued with a DAkkS calibration certificate and bear a calibration mark. Using the measurement values documented and certified in the calibration certificate, users can check and calibrate their spectrophotometers accordingly.

**Introduction**

1. **DAkkS calibration certificate**

   This certificate is an essential part of the Hellma Analytics calibration process. It ensures the accuracy and reliability of reference materials used in analytical laboratories. The certificate includes detailed information about the calibration process, measurement conditions, and the equipment used, providing users with the confidence that their reference materials are of the highest quality.

   **Important Information**

   Only if the DAkkS calibration certificate has been issued and the calibration mark has been affixed do the reference materials actually become certified reference materials.
1. INTRODUCTION

1.5 DAkkS calibration certificate

- Measurement value and smallest attributed measurement uncertainty that can be specified. This value only refers to Hellma Analytics measurements and applies solely to the company's specific measurement conditions. In justified cases, calibration certificates may also show measurement results that do not fall within the calibration laboratory's scope of accreditation. These must be clearly labeled as such on the calibration certificate.
- Notes on determining expanded measurement uncertainty.
- Measurement value and smallest attributed measurement uncertainty that can be specified. Initial measurements are not taken for filters used to determine wavelength accuracy.

1.6 Warranty

30-year manufacturer’s warranty on all Hellma Analytics reference materials

We’re confident of our quality and you can be confident of reliable measurement results!

All Hellma Analytics reference materials come with a 30-year warranty, provided that they are regularly recertified – every two years – at the Hellma Analytics calibration laboratory. Certified reference materials sent for recertification are carefully cleaned and recertified before being sent back with a new DAkkS calibration certificate and calibration mark. Damaged filters and filters that deviate significantly from nominal values are usually replaced in consultation with the customer.

Details on recertification can be found on page 18 and 19.

All of our reference materials come with a 30-year warranty, provided that they are regularly recertified (at least every two years) at the Hellma Analytics calibration laboratory.

<table>
<thead>
<tr>
<th>Method Name</th>
<th>Measurement</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method 1</td>
<td>Values</td>
<td>0.2710</td>
</tr>
<tr>
<td>Method 2</td>
<td>Values</td>
<td>0.0210</td>
</tr>
<tr>
<td>Method 3</td>
<td>Values</td>
<td>0.0030</td>
</tr>
<tr>
<td>Method 4</td>
<td>Values</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

The expanded uncertainty assigned to the measurement value is considered by multiplying the smallest uncertainty by the coverage factor k = 2. It has been determined in accordance with DAkkS-DAK-5. The value of the expanded uncertainty is within the range of values with a probability of 95%.

All Hellma Analytics reference materials come with a 30-year warranty, provided that they are regularly recertified (at least every two years) at the Hellma Analytics calibration laboratory.

30 YEARS WARRANTY
2. GLASS FILTERS

WAVELENGTH ACCURACY

2.1 Checking wavelength accuracy

APPLICATION
To measure wavelength accuracy, the filter absorbs the light beam of the spectrophotometer to a greater extent at certain wavelengths creating absorbance peaks. Ideally, any reference materials used to determine wavelength accuracy should have narrow, well-defined peaks at a variety of wavelengths in the UV and visible range.

2.1.1 Holmium glass filter

PRODUCT DESCRIPTION
The holmium glass filter 666-F1 has a range of narrow, well-defined peaks in the UV and visible range, making holmium an excellent choice for checking the wavelength scale of spectrophotometers. In comparison to filters that use holmium solution, the holmium glass filter has a somewhat weaker spectrum with fewer peaks. In the low UV range in particular, the absorbance behavior of the glass matrix is superimposed on the holmium peaks. The main advantage of using a glass filter over a liquid filter is that it is more robust.

NOTE
The positions of holmium peaks may vary slightly depending on the glass batch used. This is why Hellma Analytics certifies each holmium glass filter individually.

ARTICLE NO.
666F1-339

APPLICATION
Testing the wavelength accuracy in the UV and Vis range (279 nm to 638 nm) at a spectral bandwidth up to 2 nm.

CONTENT
Holmium glass filter with metal frame

STANDARD CERTIFICATION
Wavelengths: 279; 361; 453; 536; 638 nm
Slit width: 1 nm

POSSIBLE CERTIFICATION
Wavelengths: 279; 287; 361; 418; 445; 453; 460; 536; 638 nm
Slit width: all possible up to 2 nm

2.1.2 Didymium glass filter

PRODUCT DESCRIPTION
The didymium glass filter 666-F7W is made from material specially manufactured by Schott AG. Like holmium glass, didymium glass has a variety of characteristic peaks in the ultraviolet and visible range and is therefore typically used for checking wavelength accuracy. Its peaks are not as narrow as those of holmium glass filters, however. The filter’s absorbance behavior in the ultraviolet range also makes it suitable as an absorbance filter for checking photometric accuracy (see page 16, 666-F7A).

NOTE
The positions of didymium glass peaks may vary slightly depending on the glass batch used. This is why Hellma Analytics certifies each didymium glass filter individually.

ARTICLE NO.
666F7W-323 or 666F7-323

APPLICATION
Testing the wavelength accuracy in the UV and Vis range (329 nm to 875 nm) at a spectral bandwidth up to 2 nm.

CONTENT
Didymium glass filter with metal frame

STANDARD CERTIFICATION
Wavelengths: 329; 472; 512; 681; 875 nm
Slit width: 1 nm

POSSIBLE CERTIFICATION
Wavelengths: 302; 329; 430; 472; 482; 512; 681; 875 nm
Slit width: all possible up to 2 nm
2.2 Checking the photometric accuracy

APPLICATION
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.

2.2.1 Didymium glass filter F7A

PRODUCT DESCRIPTION
Didymium glass filter 666-F7A is made from material specially manufactured by Schott AG. The didymium glass filter’s absorbance behavior in the ultraviolet range also makes it suitable for use as an absorbance filter. Didymium glass filters are therefore suitable for checking wavelength accuracy in the UV-Vis range as well as checking photometric accuracy in the UV range.

Absorbance behavior in the UV range can be checked at 270 nm, 280 nm, 297 nm, 321 nm, and 342 nm. Filters are routinely set at a thickness that produces a nominal optical density of 0.5 Abs at 342 nm. This results in increasingly larger absorbances the shorter the wavelengths become.

NOTE
These absorbance values vary greatly depending on the glass batch used and can only be compared for filters derived from the same glass melting process. This is why all didymium glass filters are certified individually.

ARTICLE NO.
666F7A-323 or 666F7-323

APPLICATION
Testing the wavelength accuracy in the UV and Vis range (270 nm to 340 nm)

CONTENT
Didymium glass filter with metal frame

STANDARD CERTIFICATION
Photometric accuracy: approx. 0.5 to 1 Abs.
Wavelengths: 270, 280, 297, 321, 342 nm
Slit width: 1 nm

POSSIBLE CERTIFICATION
Additional possible wavelengths: from 270 to 280 nm
Slit width: all possible up to 3 nm

NOTE
These absorbance values vary greatly depending on the glass batch used and can only be compared for filters derived from the same glass melting process. This is why all didymium glass filters are certified individually.

2.2.2 Neutral density glass filter

PRODUCT DESCRIPTION
Hellma Analytics neutral density glass filters are made from filter materials produced by Schott AG, which were selected on account of their homogeneity and stability. Thanks to a relatively constant transmittance within the wavelength range of 405 nm to 800 nm, they have been used to check photometric accuracy and linearity in the visible wavelength range (>405 nm) for decades.

NOTE
If you have several Neutral density glass filters with different nominal absorbances, you can check the linearity of your absorbance scale by plotting the absorbance values measured for each wavelength against the measurement values on the DAkkS calibration certificate in a diagram.

ARTICLE NO.
666F390-25, 666F2-39, 666F201-39, 666F3-38, 666F204-37, 666F4-37, 666F202-36, 666F203-36, 666F301-361, 666F303-361

APPLICATION
Checking the photometric accuracy in the Vis range (405 nm to 890 nm)

CONTENT
Neutral density glass filter:
F390 (0.04 Abs); F2 (0.25 Abs); F201 (0.3 Abs); F3 (0.5 Abs); F204 (0.7 Abs); F4 (1.0 Abs); F202 (1.5 Abs); F203 (2.0 Abs); F301 (2.5 Abs); F303 (3.0 Abs)

STANDARD CERTIFICATION
Wavelengths:
440; 465; 546.1; 590; 635 nm
Slit width: 1 nm

POSSIBLE CERTIFICATION
All wavelengths possible from 405 to 890 nm. Also possible above 890 nm, with Hellma Analytics calibration certificate
Slit width: all possible up to 5 nm
## 2. GLASS FILTERS

### 2.3 Glass filter sets

Specifically created to meet customer requirements, Hellma Analytics glass filter sets consist of existing individual filters suitable for standard or custom validation procedures. To ensure that filters can be easily identified, the set number is engraved on each filter frame. The absorbance/peak position values measured for each filter can be found on the DAkkS calibration certificate provided.

<table>
<thead>
<tr>
<th>ARTICLE NO.</th>
<th>APPLICATION</th>
<th>CONTENT</th>
<th>STANDARD CERTIFICATION</th>
<th>POSSIBLE CERTIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>666S000</td>
<td>Complete Glass Filter Set for testing the photometric accuracy and the wavelength accuracy of the spectrophotometer</td>
<td>F1, Holmium glass filter; F2, Neutral density glass filter (0.25 Abs); F3, Neutral density glass filter (0.5 Abs); F4, Neutral density glass filter (1.0 Abs); F0, Filter frame without glass (reference filter)</td>
<td>F1, Holmium glass filter: Wavelengths accuracy: 279; 361; 453; 536; 638 nm</td>
<td>F1, Holmium glass filter: Wavelength accuracy: 279; 361; 453; 536; 638 nm</td>
</tr>
<tr>
<td>666S001</td>
<td>Glass Filter Set for checking the wavelength accuracy and the photometric accuracy of the spectrophotometer</td>
<td>F3, Neutral density glass filter (0.5 Abs); F4, Neutral density glass filter (1.0 Abs); F7, Didymium glass filter (0.5 – 1.0 Abs)</td>
<td>F3, F4 Neutral density glass filter: Photometric accuracy: Wavelengths: 440; 465; 564; 1; 590; 635 nm</td>
<td>F3, F4 Neutral density glass filter: Photometric accuracy: Wavelengths: 440; 465; 564; 1; 590; 635 nm</td>
</tr>
</tbody>
</table>

### 2.4 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 black anodized precision frames made of aluminum. 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. Anodized aluminum holders should not come into contact with acids or alkalis.

#### STORAGE

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

#### OTHER FACTORS THAT MAY INFLUENCE 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.

#### CLEANING

Dirt often accumulates on optical surfaces as a result of regular use. This is best removed using a lint-free cloth and alcohol.

#### INFLUENCE OF TEMPERATURE ON MEASUREMENTS

Temperature has a very small influence on certified measurement values, and temperatures between 20°C und 24°C fall within the measurement uncertainty stated on the calibration certificate. Measurements should therefore be taken in this range to keep any potential temperature influence on the results to a minimum.

More sets, as well as the complete product overview see page 60 onwards.
2.5 Calibration with glass filters

2.5.1 Preparations

1. Warm up the spectrophotometer until the correct operating temperature has been reached and remains constant (e.g. for one hour), taking care to observe the device manufacturer’s guidelines.

2. Make sure that you use a stable cuvette holder for 10 mm standard cuvettes to measure the filters, as this is the only way to guarantee the best positioning of the filters in the light path. Check that the holder is secure and stable in the sample compartment.

3. To begin with, carry out a baseline correction with an empty sample compartment.

4. Check that the filter is correctly positioned in the light path by first placing empty filter holder F0 in the cuvette holder. The F0 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 light source.

5. Check that the device’s display has not changed. In spectrophotometers with very large beams, the measurement beam may touch the filter frame (beam clipping). If this is the case, you will notice a change in the device’s display.

   If necessary, adjust the height of the cuvette holder until the light beam shines through the aperture unimpeded. To help, you can switch the device’s measurement beam to visible i.e. by adjusting the monochromator to 500 nm. There may be other ways of doing this depending on the device.

   If the light beam touches the sides of the aperture, adjust the horizontal position of the cuvette holder until the light beam shines through the center of the aperture. The filter frame is correctly positioned if the display values, from the zero adjustment performed in step 3 (baseline correction), do not change. In rare cases where the zero reading cannot be retained after inserting the empty filter frame and carrying out the above procedures it is permissible to re-zero the instrument with the empty filter frame in place and then continue with the filter measurements.

6. Carry out the filter measurement in a closed sample compartment as carefully as you would carry out a sample measurement (open sample compartments produce incorrect results).

7. Please note that, if you are using a diode array spectrophotometer with a stand-alone cuvette holder connected via a fiber-optic cable, extraneous light and vibrations (e.g. movement of fiber-optic cables) may impair the accuracy of measurement results.

2.5.2 Steps for checking wavelength accuracy with holmium glass or didymium glass filter

1. First, carry out the “Preparations” according to chap. 2.5.1

2. Run the scan program on your spectrophotometer, observing the guidelines in the user manual. Select a scanning range that covers all of the peaks listed on the filter’s calibration certificate.

3. Set your spectrophotometer to the measurement parameters that appear on the calibration certificate provided. Select - if possible - a slow scanning speed and a small data interval.

4. If possible, carry out a baseline correction.

5. Measurements are taken using an air blank which means, which means that the reference cuvette holder remains empty in double beam photometers, while a reference measurement is taken using the empty cuvette holder in single beam photometers.

6. Insert the holmium glass or didymium glass filter into the cuvette holder. Ensure that the filter is inserted into the holder as far as it will go, and that the filter ID can be seen from above. The filters must always be positioned in the cuvette holder in the same way, i.e. with the serial number facing the light source.

7. Start the measurement.

8. Calculate the positions of the peaks at the wavelengths stated on the calibration certificate. (Take several measurements and then use the mean of the measured values to avoid errors).

9. Compare your measurement values with the certified ones.

MEASUREMENT PARAMETERS FOR CHECKING WAVELENGTH ACCURACY

Ensure that you have selected the correct measurement parameters before scanning the absorbance curve to calculate peak positions. Incorrect parameters may distort the absorbance curve and thus shift the actual positions of peaks. Please use the settings stated on the accompanying calibration certificate. It should be noted that changing the slit width of the spectrophotometer can cause the absorption maxima to shift slightly. Ignore any influence that the spectral bandwidth from 1 nm to 2 nm has on peak positions. Peak heights, however, may vary greatly following changes to the slit width due to their narrow nature. As a result, filters for checking wavelength accuracy are usually unsuitable for checking absorbance accuracy.

“Generally speaking, filters can also be measured using a slit width that differs from the information provided on the calibration certificate. However, please note that large slit widths will prevent peaks lying close together from being resolved.”

Thomas Brenn,
Product Manager
2. GLASS FILTERS

2.5.3 Steps for checking photometric accuracy with a neutral density glass filter or a didymium glass filter (F7A)

1. First, carry out the “Preparations” according to chap. 2.5.1

2. Run the wavelength selection program on your spectrophotometer, observing the guidelines in the user manual. Select the wavelengths provided on the calibration certificate.

3. Set your spectrophotometer to the measurement parameters that appear on the calibration certificate provided.

4. Adjust to zero.

5. Measurements are taken using an air blank which means that the reference cuvette holder remains empty in double beam spectrophotometers, while a reference measurement is taken using the empty cuvette holder in single beam spectrophotometers.

6. Insert the neutral density glass or didymium glass filter into the cuvette holder. Ensure that the filter is inserted into the holder as far as it will go, and that the filter ID can be seen from above. The filters must always be positioned in the cuvette holder in the same way, i.e. with the serial number facing the light source.

7. Start the program for measuring the absorbance values at the wavelengths stated on the calibration certificate. (Take several measurements and then use the mean of the measured values to avoid errors).

8. Compare your measurement values with the certified ones.

2.5.4 Calibration with glass filters – interpreting measurement results

Reliable partners:
If you have any questions, our competence team will be pleased to assist you. For contact details see page 63

The measurement uncertainties that appear on the calibration certificate only refer to measurements conducted by Hellma Analytics and apply solely to the measurement conditions at the company (spectrophotometer used, environmental influences such as temperature, air humidity, user influence, and reference materials used).

The smallest possible measurement uncertainty can then be derived by statistically combining the measurement uncertainty stated on the calibration certificate and all of the user’s uncertainty contributions. These include the wavelength scale tolerance of the spectrophotometer used and other influences on measurement accuracy (environmental factors such as temperature, air humidity, user influence, etc.). For further literature on correctly calculating measurement uncertainty, please refer to chapter 8 of this user manual.
3. LIQUID FILTERS
WAVELENGTH ACCURACY

3.1 Checking wavelength accuracy

APPLICATION
To measure wavelength accuracy, the filter reduces the light beam of the spectrophotometer to a greater extent at certain wavelengths (peaks). Ideally, any standards used to determine wavelength accuracy should have narrow, well-defined peaks at a variety of wavelengths in the UV and visible range.

3.1.1 Holmium liquid filter

PRODUCT DESCRIPTION
The holmium liquid filter consists of a solution of holmium dissolved in perchloric acid. This filter is ideally suited to checking the wavelength accuracy of spectrophotometers in the UV and visible range. It has a spectrum with a variety of characteristic, very well-defined peaks in the range between 240 nm and 650 nm.

ARTICLE NO. 667005
APPLICATION Checking the wavelength accuracy according to Ph. Eur. in the UV and VIS range
CONTENT Holmium in perchloric acid
STANDARD CERTIFICATION Wavelengths: 241, 250, 278, 287, 333, 345, 361, 386, 416, 451, 468, 485, 536, 560 nm
POSSIBLE CERTIFICATION Wavelengths: all up to 2 nm; above peaks become indistinct

ARTICLE NO. 667025
APPLICATION Checking the wavelength accuracy according to USP <857> in the UV and VIS range
CONTENT Didymium in perchloric acid
STANDARD CERTIFICATION Wavelengths: 329, 354, 444, 469, 482, 512, 522, 575, 732, 740, 794, 801, 864 nm
POSSIBLE CERTIFICATION Slit width: all up to 2 nm

3.1.2 Didymium liquid filter

PRODUCT DESCRIPTION
The didymium liquid filter consists of praseodymium and neodymium, dissolved in perchloric acid. This filter is ideally suited for checking the wavelength accuracy of spectrophotometers in the UV and visible range. It has a spectrum with a variety of characteristic, very well-defined peaks in the range between 320 nm and 870 nm.

ARTICLE NO. 667005
APPLICATION Checking the wavelength accuracy according to Ph. Eur. in the UV and VIS range
CONTENT Holmium in perchloric acid
STANDARD CERTIFICATION Wavelengths: 241, 250, 278, 287, 333, 345, 361, 386, 416, 451, 468, 485, 536, 640 nm
POSSIBLE CERTIFICATION Slit width: all up to 2 nm

ARTICLE NO. 667025
APPLICATION Checking the wavelength accuracy according to USP <857>
CONTENT Didymium in perchloric acid
STANDARD CERTIFICATION Wavelengths: 329, 469, 575, 740, 864 nm
POSSIBLE CERTIFICATION Slit width: all up to 2 nm
3. LIQUID FILTERS

WAVELENGTH ACCURACY

3.1.3 HoDi liquid filter

PRODUCT DESCRIPTION
The HoDi liquid filter consists of a solution of holmium and didymium (praseodymium and neodymium) in perchloric acid. This filter features an especially broad wavelength spectrum and is therefore ideally suited to checking the wavelength accuracy of spectrophotometers in the UV and visible range. It has a broad spectrum with a variety of characteristic, very well-defined peaks in the range between 241 nm and 864 nm. Depending on the performance of the spectrophotometers used, up to 22 peaks can be detected at a slit width of 1 nm.

HODI LIQUID FILTER
Checking the UV-Vis wavelength accuracy
Broad wavelength spectrum from 241 – 864 nm
Two filters in one: Holmium + Didymium = HoDi

3.1.4 Rare Earth liquid filter

PRODUCT DESCRIPTION
The rare earth liquid filter consists of a solution of rare earth metals dissolved in perchloric acid. This filter is ideally suited to checking the wavelength accuracy of spectrophotometers in the low UV range. It has a spectrum with five characteristic peaks in the range from 201 nm to 252 nm.

RARE EARTH LIQUID FILTER
Specially developed for checking wavelength accuracy in the low UV range
Wavelength spectrum from 201 – 252 nm

Typical spectrum of a HoDi filter, measured at a slit width of 1 nm.

Typical spectrum of a rare earth filter, measured at a slit width of 1 nm.
3. LIQUID FILTERS
PHOTOMETRIC ACCURACY

3.2 Checking the photometric accuracy

APPLICATION
Photometric accuracy (absorbance) is measured by shining a light beam from the spectrophotometer through the inserted filter. An absorbance value (Abs) can be deduced from the light attenuation caused by the filter.

3.2.1 Potassium dichromate liquid filter for checking the photometric accuracy in accordance to Ph. Eur. and USP <857>

PRODUCT DESCRIPTION
Potassium dichromate in perchloric acid is very suitable for checking the photometric accuracy of spectrophotometers. In the UV range, the Potassium dichromate spectrum shows two characteristic peaks at 213 nm and 261 nm. The filter solutions are manufactured in strict compliance with NIST requirements and filled under controlled conditions. The cuvettes are then immediately fused to become airtight.

NOTE
As the filters are certified individually, measurement results are free from systematic errors made when preparing solutions and with regards to the optical path length of the cuvette. The measurement values of reference filter UV14 ( perchloric acid measured against an air blank) appear separately on the DAkkS calibration certificate. To check absorbance linearity, take measurements using Potassium dichromate filters with different concentrations. Plot the absorbance values measured for each filter and wavelength against the measurement values that appear on the DAkkS calibration certificate in a graph.

Typical spectrum of a Potassium dichromate solution with 60 mg/l.

ARTICLE NO.
667001, 667002, 667006, 667010, 667012, 667014, 667016, 667018, 667020, 667022, 667040, 667042, 667060, 667062, 667080, 6670100, 6670120, 6670140, 6670160, 6670180, 6670200, 6670220, 6670400, 6670420, 6670600, 6670620, 6670800, 667014 (reference filter)

APPLICATION
Checking the photometric accuracy in the UV range (235 nm to 350 nm) and Vis range (430 nm to 700 nm) with a spectral bandwidth of 2 nm or less.

CONTENT

<table>
<thead>
<tr>
<th>ARTICLE NO.</th>
<th>Wavelengths</th>
<th>UV absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV20, 20 mg/l Potassium dichromate in HClO4</td>
<td>200 – 400 nm</td>
<td>0.1 – 0.3 Abs, acc. to USP &lt;857&gt;</td>
</tr>
<tr>
<td>UV01, 60 mg/l Potassium dichromate in HClO4</td>
<td>200 – 400 nm</td>
<td>0.2 – 0.6 Abs, acc. to USP &lt;857&gt;</td>
</tr>
<tr>
<td>UV02, 60 mg/l Potassium dichromate in HClO4</td>
<td>200 – 400 nm</td>
<td>0.3 – 0.7 Abs, acc. to USP &lt;857&gt; and Ph. Eur.</td>
</tr>
<tr>
<td>UV03, 80 mg/l Potassium dichromate in HClO4</td>
<td>200 – 400 nm</td>
<td>0.4 – 1.2 Abs, acc. to USP &lt;857&gt;</td>
</tr>
<tr>
<td>UV04, 100 mg/l Potassium dichromate in HClO4</td>
<td>200 – 400 nm</td>
<td>0.5 – 1.5 Abs, acc. to USP &lt;857&gt;</td>
</tr>
<tr>
<td>UV05, 120 mg/l Potassium dichromate in HClO4</td>
<td>200 – 400 nm</td>
<td>0.6 – 1.8 Abs, acc. to USP &lt;857&gt;</td>
</tr>
<tr>
<td>UV06, 140 mg/l Potassium dichromate in HClO4</td>
<td>200 – 400 nm</td>
<td>0.7 – 2.0 Abs, acc. to USP &lt;857&gt;</td>
</tr>
<tr>
<td>UV07, 180 mg/l Potassium dichromate in HClO4</td>
<td>200 – 400 nm</td>
<td>0.9 – 2.6 Abs, acc. to USP &lt;857&gt;</td>
</tr>
<tr>
<td>UV08, 200 mg/l Potassium dichromate in HClO4</td>
<td>200 – 400 nm</td>
<td>1.1 – 3.0 Abs, acc. to USP &lt;857&gt;</td>
</tr>
<tr>
<td>UV09, 200 mg/l Potassium dichromate in HClO4</td>
<td>200 – 400 nm</td>
<td>1.0 – 3.0 Abs, acc. to USP &lt;857&gt;</td>
</tr>
<tr>
<td>UV10, 400 mg/l Potassium dichromate in HClO4</td>
<td>200 – 400 nm</td>
<td>2.0 – 4.0 Abs, acc. to USP &lt;857&gt;</td>
</tr>
</tbody>
</table>

STANDARD CERTIFICATION

- UV20 to UV0200 at wavelengths: 213 nm and 261 nm at a spectral bandwidth of 2 nm or less.
- UV600 at wavelengths: 430 nm (Vis range)
- Slit width: 2 nm

POSSIBLE CERTIFICATION

- Wavelengths: as given above
- Slit width: all up to 2 nm

3.2.2 Niacin liquid filter

PRODUCT DESCRIPTION
Niacin (nicotinic acid) in hydrochloric acid is highly suitable for checking the photometric accuracy of spectrophotometers. The niacin spectrum shows in the UV range two characteristic peaks at 213 nm and 261 nm. The niacin filter solutions are filled and immediately fused under controlled conditions to become permanently airtight.

NOTE
The individually measured absorbance values are free from any systematic errors. The measured data of the reference filter UV59 (hydrochloric acid measured against air blank) appear separately on the DAkkS calibration certificate. To check absorbance linearity, perform the measurement with niacin filters of different concentrations. List the measured absorbance values for each filter and each wavelength in a diagram against the values on the DAkkS calibration certificate.

Typical scanlines of Niacin liquid filters, measured at a slit width of 1 nm.

ARTICLE NO.
667001, 667002, 667006, 6670100, 6670120, 6670140, 6670160, 6670180, 6670200, 6670220, 6670400, 6670420, 6670600, 6670620, 6670800 (reference filter)

APPLICATION
Checking the photometric accuracy in UV range (213 nm and 261 nm) at a spectral bandwidth of 2 nm or less.

CONTENT

<table>
<thead>
<tr>
<th>ARTICLE NO.</th>
<th>Wavelengths</th>
<th>UV absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV51, 6 mg/l Niacin in HCl</td>
<td>200 – 400 nm</td>
<td>0.25 – 0.6 Abs</td>
</tr>
<tr>
<td>UV52, 12 mg/l Niacin in HCl</td>
<td>200 – 400 nm</td>
<td>0.50 Abs</td>
</tr>
<tr>
<td>UV53, 18 mg/l Niacin in HCl</td>
<td>200 – 400 nm</td>
<td>0.75 Abs</td>
</tr>
<tr>
<td>UV54, 24 mg/l Niacin in HCl</td>
<td>200 – 400 nm</td>
<td>1.00 Abs</td>
</tr>
<tr>
<td>UV59, hydrochloric acid (HCl), (Reference filter)</td>
<td>200 – 400 nm</td>
<td>1.00 Abs</td>
</tr>
</tbody>
</table>

STANDARD CERTIFICATION

- UV51 to UV54 at wavelengths: 213 nm and 261 nm at a spectral bandwidth of 2 nm or less.
- Slit width: 2 nm

POSSIBLE CERTIFICATION

- Wavelengths: as given above
- Slit width: all up to 2 nm

More sets, as well as the complete product overview see page 60 onwards.
3. LIQUID FILTERS

3.3 Checking for stray light

APPLICATION
In a spectrophotometer, stray light is light that passes by the sample and falls directly on the detector. This can lead to incorrect measurement results. Stray light may be caused by scattering or diffraction, poor optical alignment, the use of incorrect or damaged cuvettes, incorrectly fitted sampling accessories or damaged seals around a light-tight sample chamber. Stray light is problematic, as it reduces the range of measurable absorbance and impairs the linearity between concentration and absorbance. Cut-off filters (filters with a strictly defined spectrum) are required to check the device for stray light.

Due to their strictly defined spectrum, Potassium Chloride filters, Sodium Iodide filters, Niacin and Sodium Nitrite filters are ideally suited to qualifying the stray light level of spectrophotometers. Due to their strictly defined spectrum, Potassium Chloride filters are required to check the device for stray light. Hellma Analytics stray light filters do not allow light to pass through them below a certain wavelength (cut-off wavelength). Any transmittance values displayed in the cut-off wavelength range therefore represent stray light.

According to the Ph. Eur method, the measurement is made against the reference filter which is filled with water.

The steps are the same for all stray light filters.

3.3.1 Checking for stray light – measurement in accordance with Ph. Eur.

PRODUCT DESCRIPTION
Hellma Analytics stray light filters do not allow light to pass through them below a certain wavelength (cut-off wavelength). Any transmittance values displayed in the cut-off wavelength range therefore represent stray light. According to the Ph. Eur method, the measurement is made against the reference filter which is filled with water.

Measurement in accordance with Ph. Eur.

3.3.2 Checking for stray light – measurement in accordance with USP <857>

PRODUCT DESCRIPTION
The new chapter <857> of the USP describes a new measuring method for checking for stray light. "When using a 5 mm path length cell (filled with the same filter) as the reference cell and then measuring the 10 mm cell over the required spectral range, analysts can calculate the stray light value from the observed maximum absorbance using the formula" (Quoted from USP <857>).

This means that in a single-beam photometer, first of all, the reference filter with 5 mm path length (filled with the same solution) is measured. The 10 mm stray light filter is then measured for the same required spectral range.

In a double-beam spectrophotometer, the stray light filter with 10 mm path length is measured against the reference filter filled with the same solution with 5 mm path length.

The stray light value can now be calculated from the absorbance maximum obtained, using the following formula: 

$$ S_{\lambda} = 0.25 \times 10^{-2} A_{\lambda} $$

The following acceptance criteria apply: 

$$ A_{\lambda} \geq 0.7 \text{ Abs} \quad \text{and} \quad S_{\lambda} \leq 0.01 $$

$$ A_{\lambda} = \text{absorbance measured at peak maximum at wavelength } \lambda $$

$$ S_{\lambda} = \text{stray light value calculated at wavelength } \lambda $$

The measurement indicated on the calibration certificate for the wavelength at the peak maximum refers only to the measurement taken with the UV-Vis-NIR spectrophotometer shown on the calibration certificate. This wavelength is instrument-dependent due to the different optical components installed and the resulting differences in performance, and so it is not applicable to other UV-Vis-NIR spectrophotometers. The indicated measurement of the wavelength at the peak maximum is not suitable for checking the wavelength scale.

NOTE
Experience from everyday use shows that the values obtained using this stray light measurement method are extremely instrument-dependent, i.e. the wavelength of the peak position varies depending on the type of instrument and its performance. The important thing for the user to know is that, when using this testing method, the maximum absorbance measured in the testing range is the deciding factor and should be ≥ 0.7 Abs.
3. LIQUID FILTERS

STRAY LIGHT

3.3.2 Checking for stray light measurement in accordance with USP <857>

The Hellma Analytics USP stray light filter sets each consist of a 10 mm stray light filter and a 5 mm reference filter, both filled with the same solution. These filter sets meet the criteria of USP <857> and are therefore ideal for qualifying the stray light level of spectrophotometers in accordance with the requirements of the USP. The procedure for determining the stray light value is the same for all stray light filter sets, the difference lies in the cut-off range for each set.

ARTICLE NO. 667006/667006H, 667010/667010H, 667011/667011H, 667019/667019H
Letter H indicates the reference filter with 5 mm path length

APPLICATION Checking for stray light in the UV range, measurement acc. to USP <857>

CONTENT UV6, Toluene in hexane, acc. to USP <857>
UV9, hexane (reference filter)

STANDARD CERTIFICATION UV6: Cut-Off approx. 198 nm*, spectral range 190 – 205 nm
UV9: Cut-Off approx. 259 nm*, spectral range 210 – 259 nm

POSSIBLE CERTIFICATION Wavelength: fixed Possible slit widths: 1 to 5 nm

* depending on the device

EXPERTS TIP:
Benjamin Brix, Biological-technical assistant

NOTES
A spectrophotometer's spectral resolution is very closely connected to the correct slit width setting and characterized by its ability to resolve (recognize) two very closely related peaks. The smaller the slit and corresponding spectral bandwidth, the higher the resolution. As a rule of thumb, the slit width should be no more than 10 % of the peak width at half maximum in order to be able to determine its absorbance with an accuracy of 99.5 %. Two peaks are deemed to be resolved separately if the minimum absorbance between them amounts to less than 80 % of the peak maximum. If the spectrophotometer's spectral resolution is impaired, two different peaks will be shown as a combined peak, leading to inaccurate measurement results.

3. LIQUID FILTERS

SPECTRAL RESOLUTION

3.4 Checking the spectral resolution

APPLICATION Regularly checking the spectral resolution of spectrophotometers ensures, for example, that neighbouring peaks are resolved and not superimposed on the peaks of bordering wavelengths. This also prevents absorbance errors.

PRODUCT DESCRIPTION
The Toluene in hexane liquid filter has a prominent point in its spectrum, which is excellent for determining the spectral resolution and/or actual slit width of spectrophotometers in compliance with the European Pharmacopeia, and also the USP <857>.

For improved handling of the reference filters with 5 mm light path, our USP stray light reference filters have the same external dimensions as a cuvette with 10 mm path length. The reference filters with 5 mm path length are marked with the letter H. There is no need for an additional spacer.

ARTICLE NO. 667006, 667009; set: 667200

APPLICATION Testing the resolution in accordance to Ph. Eur. and USP <857>

CONTENT UV6, Toluene in hexane
UV9, hexane (reference filter)

STANDARD CERTIFICATION Wavelengths: scan from 265 to 270 nm
Slit width: 0.5; 1.0; 1.5; 2.0; 3.0 nm

POSSIBLE CERTIFICATION Wavelength: fixed Possible slit widths: all between 0.5 to 3 nm

Typical spectrum of the toluene liquid filter, measured with different slit widths.

“If the spectrophotometer’s spectral resolution is impaired, two peaks may be shown as a combined peak, for example, leading to inaccurate measurement results.”

Benjamin Brix, Biological-technical assistant
3. LIQUID FILTERS

SETS

3.5 Liquid filter sets

3.5.1 Set in accordance to Ph. Eur.

The complete liquid filter set – art. no. 667003 – was compiled on the basis of European Pharmacopeia requirements and contains all filters required to carry out a complete spectrophotometer check.

- Checking wavelength accuracy (UV6)
- Checking photometric accuracy (UV60, UV600, UV14)
- Checking stray light (UV1 and UV12)
- Checking spectral resolution (UV6 and UV9)

Measurement in accordance with Ph. Eur.

All liquid filters consist of reference materials that are filled into precision Hellma cuvettes made of high performance quartz glass. These cuvettes are permanently sealed, and the complete set is delivered in a high-quality storage box. To ensure easy identification, each filter is engraved with its serial number. The calibration values measured for each filter can be found on the DAKKS and Hellma Analytics calibration certificates provided.

Measurement in accordance with USP <857>

3.5.2 Set in accordance to USP <857>

The USP basic filter set – art. no. 667857 – was compiled based on the specifications of the USP. The set contains a basic selection of filters for all parameters to be checked in the USP <857>. Depending on the intended operational range, this basic set can be supplemented accordingly by the user.

<table>
<thead>
<tr>
<th>ARTICLE NO.</th>
<th>667857</th>
</tr>
</thead>
<tbody>
<tr>
<td>APPLICATION</td>
<td>Basic set for checking spectrophotometers in accordance to USP &lt;857&gt;</td>
</tr>
</tbody>
</table>
| CONTENT | F2, Neutral density glass filter with 0.25 Abs  
F3, Neutral density glass filter with 0.5 Abs  
F4, Neutral density glass filter with 1.0 Abs  
UV60, 60 mg/l Potassium dichromate in perchloric acid with 0.3 to 0.9 Abs  
UV14, Perchloric acid (reference filter) |
| STANDARD CERTIFICATION | UV5, Holmium in perchloric acid  
UV25, Didymium in perchloric acid  
UV11, Sodium nitrite in pure water with 10 mm path length (reference filter)  
UV6, Toluene in hexane  
UV9, Hexane (reference filter) |
| POSSIBLE CERTIFICATION | F2, F3, F4 at wavelengths: 640, 645, 646, 5, 598, 635 nm; slit width: 1 nm  
UV60 at wavelengths: 230, 257, 313, 350 nm; slit width: 1 nm  
UV14 at wavelengths: 440, 465, 546.1, 590, 635 nm; slit width: 1 nm  
F2, F3, F4, Neutral density glass filters with 0.1, 0.5, 1.0, 1.5, 2.0, 3.0 Abs |
| ALL WAVELENGTHS | All wavelengths up to 5 nm; slit width: 0.2 nm  
Scan area is fixed; slit width: 0.5 to 3 nm  
No further wavelengths possible; slit width: all up to 2 nm  
UV60 at wavelengths: 230; 257; 313; 350 nm; slit width: 1 nm |
| POSSIBLE CERTIFICATION | F2, F3, F4; wavelengths: free selectable between 605 – 890 nm; slit width: all up to 5 nm  
UV60; wavelengths: 1; slit width: all up to 2 nm  
UV11/UV14 wavelengths: 10; slit width: all up to 5 nm  
UV5; no further wavelengths possible; slit width: all up to 2 nm  
UV25; wavelengths: 605; 610; 615; 620; 625; 630 nm; slit width: all up to 2 nm  
UV60/UV14; scan area is fixed; slit width: all between 0.5 to 3 nm |
3. LIQUID FILTERS

3.6 General usage guidelines

Liquid filters bear a marking on one side showing the chemical formula of the substance contained in the cuvette. If a filter breaks, please observe the codes of conduct and safety instructions that apply to this substance. This information can be found in the safety instructions. Up-to-date safety instructions for all substances used to manufacture liquid filters are available at www.hellma-analytics.com/download

STORAGE
After use, we strongly recommend storing the filters at room temperature, in their storage box, and in a dry, dust-free area. Liquid filters must not be exposed to temperatures below 4°C or above 40°C. This also applies when transporting and delivering liquid filters for recertification. Especially during the winter months, attention must be paid to adequate packaging.

OTHER FACTORS THAT MAY INFLUENCE MEASUREMENTS
Dirt (e.g. fingerprints) and dust on, or damage (scratches, corrosion) to polished 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 caps or matt surfaces.

CLEANING
Dirt often accumulates on optical surfaces as a result of regular use. This is best removed using a lint-free cloth and alcohol.

INFLUENCE OF TEMPERATURE ON MEASUREMENTS
Temperature has a very small influence on certified measurement values, and temperatures of between 20°C und 24°C fall within the measurement uncertainty stated on the calibration certificate. Measurements should therefore be taken in this range to keep any potential temperature influence on the results to a minimum.

3.7 Calibration with liquid filters (wavelength accuracy and photometric accuracy)

3.7.1 Preparations

1. Warm up the spectrophotometer until the correct operating temperature has been reached and remains constant (e.g. for one hour). Please also note the instructions of your instrument manufacturer.
2. Make sure that you use a stable cuvette holder for 10 mm standard cuvettes to measure the liquid filters, as this is the only way to guarantee the best positioning of the filters in the light path. Check that the holder is secure and stable in the sample compartment.
3. The filters should always be positioned in the cuvette holders in the same way, i.e. with the Hellma lettering facing the light source. The light beam must pass through the part of the filter filled with liquid (solution).
4. Carry out the filter measurement in a closed sample compartment as carefully as you would carry out a sample measurement (open sample compartments produce incorrect results).
5. Please note that, if you are using a diode array spectrophotometer with a stand-alone cuvette holder connected via a fiber-optic cable, extraneous light and vibrations (e.g. movement of fiber-optic cables) may also impair the accuracy of measurement results.

FOR INFORMATION

Always take great care when placing liquid filters in the sample holder of your spectrophotometer. Wherever possible, only touch filters by their caps or matt sides. Take care not to touch the polished surfaces. Grease on the fingers may cause a greasy film on the polished surfaces, which may affect the measurement results. The filters are fragile and should be handled with the utmost care.
3. LIQUID FILTERS

3.7.2 Steps for checking wavelength accuracy with Holmium, Didymium, Rare Earth or HoDi liquid filter

1. First of all, follow the ‘steps to take before performing calibration with liquid filters’ (see chapter 3.7).
2. Run the scan program on your spectrophotometer, observing the guidelines in the user manual. Select a scanning range that covers all of the peaks listed on the filter’s calibration certificate.
3. Set your spectrophotometer to the measurement parameters that appear on the calibration certificate provided. Select the slowest scanning speed and a small data interval.
4. If possible, carry out a baseline correction.
5. Measurements are taken against the Perchloric Acid blank UV14, which means that the reference cuvette holder remains empty in double beam photometers, while a reference measurement is taken using the empty cuvette holder in single beam photometers.
6. Insert the holmium liquid filter into the cuvette holder, observing the general usage guidelines for liquid filters. The filters should always be positioned in the cuvette holders in the same way, i.e. with the Hellma lettering facing the light source.
7. Start the measurement.
8. Calculate the positions of the peaks at the wavelengths stated on the calibration certificate. (Take several measurements and then use the mean of the measured values to avoid errors).
9. Compare your measurement values with the certified ones, suitable for this purpose is, for example, a control chart (see page 41).

3.7.3 Steps for checking photometric accuracy with Potassium Dichromate or Niacin liquid filter

1. First of all, follow the ‘steps to take before performing calibration with liquid filters’ (see chapter 3.7).
2. Run the scan program on your spectrophotometer, observing the guidelines in the user manual. Select a scanning range that covers all of the peaks listed on the filter’s calibration certificate.
3. Set your spectrophotometer to the measurement parameters that appear on the calibration certificate provided. If this is not possible, please ensure that the integration time is not too short.
4. If possible, carry out a baseline correction.
5. The measurements are usually carried out against a filled with perchloric acid or hydrochloric acid reference filter. Observe the general handling instructions for liquid filters. The filters should always be placed in the same orientation in the cuvette holder, e.g. always with the Hellma logo to the light source.
6. Measurement in a single-beam spectrophotometer: Carefully insert the supplied perchloric acid or hydrochloric acid reference filter into the cuvette holder. Start the measurement. Then measure the certified reference material, which contains potassium dichromate dissolved in perchloric acid or niacin in hydrochloric acid. Then subtract the values of the reference measurement from the values of the measurement of the certified reference material.
7. Measurement in a two-beam spectrophotometer: Put the certified reference material with dissolved potassium dichromate in perchloric acid or niacin in hydrochloric acid, carefully into the sample holder and the perchloric acid or hydrochloric acid reference filter in the reference sample holder.
8. Start the program for measuring absorbance values at the wavelengths indicated on the calibration certificate. Take several measurements and average your measured values to avoid errors.
9. Compare your measurement values with the certified ones, suitable for this purpose is, for example, a control chart (see page 41).

MEASUREMENT PARAMETERS FOR CHECKING WAVELENGTH ACCURACY

Ensure that you have selected the correct measurement parameters before plotting the absorbance curve to calculate peak positions. Incorrect parameters may distort the absorbance curve and thus shift the actual positions of peaks. Please use the settings stated on the accompanying calibration certificate. It should be noted that changing the slit width of the spectrophotometer can cause the absorption maxima to shift slightly. Ignore any influence that the spectral bandwidth from 1 nm to 2 nm has on peak positions. Peak heights, however, may vary greatly following changes to the slit width due to their narrow nature. As a result, filters for checking wavelength accuracy are usually unsuitable for checking absorbance accuracy.

EXPERTS TIP:
Carola Steinger, Chemistry lab technician

“Generally speaking, filters can also be measured using a slit width that differs from the information provided on the calibration certificate. However, please note that large slit widths will prevent peaks lying close together from being resolved.”
3. LIQUID FILTERS

3.7.4 Calibration with liquid filters – interpreting the measurement results (wavelength accuracy and photometric accuracy)

MEASUREMENT PARAMETERS FOR CHECKING PHOTOMETRIC ACCURACY

As the difference between the maxima and minima in the absorbance spectrum is relatively large, the Potassium dichromate liquid filters or the Niacin filters may also be measured with a slit width that differs from the one on the calibration certificate. However, please note that using large slit widths (> 2 nm) may result in slight deviations from the values stated on the calibration certificate. In cases of doubt, it is therefore advisable to choose the slit width quoted on the calibration certificate. We recommend taking several measurements and then using the mean value to avoid errors during evaluation.

INTERPRETING THE MEASUREMENT RESULTS OF LIQUID FILTERS FOR CHECKING PHOTOMETRIC AND WAVELENGTH ACCURACY

The measurement uncertainties that appear on the calibration certificate only refer to measurements conducted by Hellma Analytics and apply solely to the measurement conditions at the company (spectrophotometer used, environmental influences such as temperature, air humidity, user influence, and reference materials used).

The smallest possible measurement uncertainty that can be achieved by the user can then be derived by statistically combining the measurement uncertainty stated on the calibration certificate plus all the user’s uncertainty contributions, such as the wavelength scale tolerance of the spectrophotometer used and other influences on measurement accuracy (environmental factors such as temperature, air humidity, user influence, etc.). For further literature on correctly calculating measurement uncertainty, please refer to chapter 8 of this user manual.

WAVELENGTH ACCURACY

PHOTOMETRIC ACCURACY

MEASUREMENT PARAMETERS FOR CHECKING PHOTOMETRIC AND WAVELENGTH ACCURACY

The measurement uncertainties that appear on the calibration certificate only refer to measurements conducted by Hellma Analytics and apply solely to the measurement conditions at the company (spectrophotometer used, environmental influences such as temperature, air humidity, user influence, and reference materials used).

The measurement uncertainties that appear on the calibration certificate plus all the user’s uncertainty contributions, such as the wavelength scale tolerance of the spectrophotometer used and other influences on measurement accuracy (environmental factors such as temperature, air humidity, user influence, etc.). For further literature on correctly calculating measurement uncertainty, please refer to chapter 8 of this user manual.

Control chart for certified reference materials

In order to achieve exact measurement results, it is important to test the spectrophotometer at regular intervals and to document the measurement results achieved. The results can be documented using control charts, for example, which also display the measurement values graphically. One example of a type of control chart is the target value chart used below.

Here, the value given on the Hellma Analytics calibration certificate is set as the target value. As the exclusion limit, you should use the measurement uncertainty you have determined (measurement uncertainty from the calibration certificate plus own measurement uncertainty), i.e. all measured values must lie within the margin of measurement uncertainty in order to avoid an out-of-control situation.

To help you in the analysis, you can download an example control chart template from our website: http://www.hellma-analytics.com/control-charts

Documentation with target value chart

“Documenting the measurement results on the target value chart gives a valuable overview and helps to quickly identify trends and deviations.”

Birgit Kehl,
Compliance Representative Calibration Laboratory
3. LIQUID FILTERS

Measurement in accordance with Ph. Eur.

3.8 Calibration with liquid filters (stray light and spectral resolution)

3.8.1 Steps for checking the stray light level in accordance to Ph. Eur. + interpretation

1. First of all, follow the ‘steps to take before performing calibration with liquid filters’.
2. Run the scan program on your spectrophotometer, observing the guidelines in the user manual. Select a scanning range that covers all of the values listed on the filter’s calibration certificate.
3. Set your spectrophotometer to the measurement parameters quoted on the calibration certificate provided.
4. Set the spectrophotometer to a wavelength of approx. 20 nm above the cut-off wavelength for the stray light filter used (for Potassium chloride (UV1), for example, start at 220 nm) and scan down to the wavelength for which you wish to determine the stray light level.
5. If possible, carry out a baseline correction.
6. The measurement is usually carried out against a reference filter (UV12) filled with water (in the case of acetone measurement against air). Please note the general guidelines for liquid filters. The filters should always be positioned in the cuvette holders in the same way, i.e. with the Hellma Analytics lettering facing the light source.
7. Measurements in a single beam spectrophotometer: Carefully insert the reference filter provided into the cuvette holder. Start the measurement. Next, measure the certified reference material. Then subtract the reference measurement values from the measurement values of the certified reference material.
8. Measurements in a double beam spectrophotometer: Carefully insert the certified reference material into the sample holder and the reference filter into the reference sample holder. Start the measurement.
9. Scan down to the wavelength for which you wish to determine the stray light level.
10. The light level (remaining transmittance value) measured below the cut-off wavelength represents stray light.

FOR INFORMATION
You can check the lower absorbance range of your spectrophotometer using reference filter 667-UV12, which is filled with ultrapure water. The filter’s absorbance characteristics from 200 nm to NIR are practically only determined by the reflection losses on the two air/glass surfaces. You can check your device’s display at very low absorbance values against the certified values at 198 nm, 200 nm, 300 nm and 400 nm. If your results differ significantly from the certified values, particularly if the measured values are smaller than 0.02 A, you should contact the service engineer.

NOTE:
Interpreting measurement results when checking for stray light. To estimate the sample measurement error due to stray light, compare the calculated stray light level to the signal strength from the sample measurement. For example, a stray light value of 0.1% transmittance and a sample with an absorbance of around 1 Abs would equate to a measurement error due to stray light of around 0.4%. If you have calculated a stray light level that is considerably higher than the level stated in the device specifications, check whether extraneous light could have interfered with this result. If you can rule out extraneous light, please contact a service technician.

MEASUREMENT PARAMETERS FOR CHECKING STRAY LIGHT LEVEL
For a realistic calculation of the stray light level, choose a filter with a cut-off wavelength as close above the required wavelength as possible. This enables the stray light test to be carried out at the wavelength at which the stray light filter can fully absorb light. The remaining transmittance displayed by the device at the measurement wavelength represents the stray light level. Since this value differs depending on the properties of the measuring system, filters can only be certified with regard to their suitability for use as a stray light filter. Certification therefore demonstrates that filters have virtually full absorbance in the measuring range and steep peaks at high transmittance values.
### 3.8.2 Procedure for checking the stray light level according to USP <857> + Interpretation

1. First, carry out the “Steps to take before performing calibration with liquid filters” according to Chap. 3.7.

2. Run the scan program on your spectrophotometer, observing the guidelines in the user manual. Select the limits of the scanning range so that all values listed on the filter’s calibration certificate are recorded.

3. If possible, set your spectrophotometer to the measurement parameters quoted on the calibration certificate provided.

4. Set the spectrophotometer to a wavelength of approx. 20 nm above the cut-off wavelength for the stray light filter used in each case with 10 mm path length (for Potassium chloride (UV1), for example, start at 220 nm) and scan to approx. 20 nm below the cut-off wavelength.

5. If possible, carry out a baseline correction.

6. The measurement is carried out against a reference filter with 5 mm path length, filled with the same solution. Follow the general usage guidelines for liquid filters. The filters should always be positioned in the cuvette holders in the same way, e.g. always with the Hellma lettering facing the light source.

7. Measurements in a single-beam spectrophotometer: Carefully insert the reference filter provided, with 5 mm path length, into the cuvette holder. Start the measurement. Next, measure the filter with 10 mm path length, filled with the same solution.

8. Measurements in a double-beam spectrophotometer: Carefully insert the filter with 10 mm path length into the sample holder, and the reference filter with 5 mm path length, filled with the same solution, into the reference sample holder. Start the measurement.

9. Scan a range of 20 nm around the cut-off.

10. Record the maximum absorbance value measured at wavelength \( \lambda \) (= \( A_\lambda \))

11. Check whether the recorded absorbance value is \( \geq 0.7 \) Abs.

12. Next, use the following formula to calculate the stray light level:

\[
S_{\lambda} = 0.25 \times 10^{-2}A_{\lambda}
\]

\( S_{\lambda} \) is the stray light value calculated for wavelength \( \lambda \).

\( A_{\lambda} \) = maximum absorbance value measured at wavelength \( \lambda \)

13. Check whether \( S_{\lambda} \leq 0.01 \).

---

**MEASUREMENT PARAMETERS FOR CHECKING STRAY LIGHT LEVEL**

To enable a realistic estimate of the stray light level, choose a filter set with a cut-off wavelength as close above the required wavelength as possible.

The stray light test is then carried out with the appropriate filter set, consisting of a stray light filter with 10 mm path length and the associated reference filter with 5 mm path length, both filters being filled with the same solution.

The stray light level \( S_{\lambda} \) calculated using the formula corresponds to the stray light level of the device at the measurement wavelength. Since this value as well as the location of the peak maximum differ depending on the properties of the measuring system, the filter set can only be certified with regard to its suitability for use as a stray light filter in accordance with USP <857>.

---

**INTERPRETING MEASUREMENT RESULTS WHEN CHECKING FOR STRAY LIGHT**

If the stray light level you have observed does not correspond to the default value criteria of USP <857>, i.e. the absorbance at the peak maximum is \( \geq 0.7 \) Abs and the stray light value calculated for the wavelength of the peak maximum \( S_{\lambda} \) is \( \geq 0.01 \), first check whether your result was caused by extraneous light. If you can rule out extraneous light, please contact one of your device manufacturer’s service technicians.
MEASUREMENT PARAMETERS WHEN CHECKING SPECTRAL RESOLUTION
When measuring spectral resolution, the liquid filter absorbs the light beam from the spectrophotometer to significantly different extents in a narrow wavelength range (5 nm). The filter will show a clear maximum and minimum within the narrow range. After placing the liquid filter in the spectrophotometer, run the scan program in the defined wavelength range and divide the maximum peak measured at $\lambda_{\text{max}} = 269$ nm by the minimum peak measured at $\lambda_{\text{min}} = 266$ nm. The resulting ratio represents the absorbance ratio, which is directly linked to the slit width. If the ratio is considerably lower (e.g. 15%), please contact the device manufacturer.

Please note, however, that the result also depends on the measurement conditions. Therefore, please make sure that you select a sufficiently long integration time, particularly if using a small slit width.

INTERPRETING MEASUREMENT RESULTS WHEN CHECKING SPECTRAL RESOLUTION
Regulatory codes or internal applications and measuring procedures may place requirements on the ratios that must be achieved. In addition, comparing calculated ratios with certified values may provide an indication of the actual slit width of the device used.

OVERVIEW: ABSORBANCE RATIO OF MAXIMUM/MINIMUM PEAK IN RELATION TO SLIT WIDTH

<table>
<thead>
<tr>
<th>SLIT WIDTH</th>
<th>ABSORBANCE RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>2.2</td>
</tr>
<tr>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>2.0</td>
<td>1.4</td>
</tr>
<tr>
<td>3.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

(Inc. Standards and Best Practice in Absorption Spectrometry, edited by C. Burgess & T. Friel)

FOR INFORMATION
Please note that the filter set for determining spectral resolution does not fall within our scope of accreditation, and therefore cannot be issued with a DAkkS calibration certificate or calibration mark.
4. REFERENCE PLATES

PHOTOMETRIC ACCURACY

4.1 Checking the photometric accuracy

APPLICATION
The Hellma Analytics reference plate 666R013 can be used to check the photometric accuracy of microplate readers.

PRODUCT DESCRIPTION
The reference plate dimensions are equivalent to a 96-well microplate with a diameter of 6.6 mm per window (H 14.5 mm x D 125 mm x L 85.5 mm). Each of the five Neutral density glass filters (columns 3 – 12) in the reference plate can measure the absorbance value for 16 windows. The other 16 windows do not contain glass (column 1 + 2) and serve as references.

NOTE
The reference plate has five Neutral density glass filters with different nominal absorbance values, allowing you to check the linearity of your absorbance scale by plotting the absorbance values measured for each wavelength against the measurement values on the DAkkS calibration certificate in a diagram.

ARTICLE NO. 666R013
APPLICATION Reference plate for microplate readers for testing the photometric accuracy
CONTENT
Neutral density glass filter (0.25 Abs); column 3 + 4
Neutral density glass filter (0.5 Abs); column 5 + 6
Neutral density glass filter (1.0 Abs); column 7 + 8
Neutral density glass filter (1.5 Abs); column 9 + 10
Neutral density glass filter (2.5 Abs); column 11 + 12
(column 1 + 2 without glass (reference filter))
STANDARD CERTIFICATION Photometric accuracy certified at wavelengths: 405; 450; 490; 650 nm; at 8 points in a column
Slit width: 1 nm
POSSIBLE CERTIFICATION Wavelengths: all possible from 405 to 890 nm
Slit widths: all possible up to 5 nm

4.2 Checking photometric and wavelength accuracy

APPLICATION
The Hellma Analytics reference plate 666R113 can be used to check the photometric and wavelength accuracy of microplate readers.

PRODUCT DESCRIPTION
The reference plate dimensions are equivalent to a 96-well microplate with a diameter of 6.6 mm per window (H 14.5 mm x D 125 mm x L 85.5 mm). Each of the four Neutral density glass filters used (columns 3 – 10) can measure the absorbance value for 16 windows. Holmium glass filter (column 11+12) are used to test the wavelength accuracy in 16 windows while a further 16 windows (columns 1+2) do not contain glass and serve as references.

NOTE
Filters are routinely set at a thickness that produces the indicated nominal optical density (ranging from 0.04 – 3.0 Abs) at 546.1 nm. This results in increasingly larger absorbances the shorter the wavelengths become.

ARTICLE NO. 666R113
APPLICATION Reference plate for microplate readers for testing the photometric and wavelength accuracy
CONTENT
Neutral density glass filter (0.5 Abs), column 3+4
Neutral density glass filter (1.0 Abs), column 5+6
Neutral density glass filter (1.5 Abs), column 7+8
Neutral density glass filter (2.0 Abs), column 9+10
Holmium glass filter, column 11+12
(column 1 + 2 without glass (reference filter))
STANDARD CERTIFICATION Photometric accuracy certified at 8 points in column at wavelengths: 405; 450; 490; 650 nm
Wavelength accuracy certified at:
279; 361; 453; 536; 638 nm
Slit width: 1 nm
POSSIBLE CERTIFICATION Photometric accuracy:
Wavelengths: all possible from 405 to 890 nm
Slit widths: all possible up to 5 nm
Wavelength accuracy:
Wavelengths: all possible above 890 nm
Slit widths: all up to 2 nm recommended.
4. REFERENCE PLATES

4.3 General usage guidelines for reference plates

Reference plates are made of glass doped with metal ions or rare earth metals, which is annealed and assembled in black anodized precision frames made of aluminum. They are designed to fit into all microplate readers. To ensure easy identification, each reference plate is engraved with the reference plate 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. Anodized aluminum frames should not come into contact with acids or alkalies.

STORAGE

After use, we recommend storing reference plates at room temperature, in their packaging, and in a dry, dust-free area.

OTHER FACTORS THAT MAY INFLUENCE MEASUREMENTS

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

CLEANING

Dirt often accumulates on optical surfaces as a result of regular use. This is best removed using a lint-free cloth and alcohol.

INFLUENCE OF TEMPERATURE ON MEASUREMENTS

Temperature has a very small influence on certified measurement values. Measurements taken at temperatures between 20°C und 24°C fall within the measurement uncertainty stated on the calibration certificate. Measurements should therefore be taken in this range to keep any potential temperature influence on the results to a minimum.

4.4 Calibration with reference plates

4.4.1 Preparations

1. Warm up the microplate reader until the correct operating temperature has been reached and remains constant (e.g. for one hour), taking care to observe the device manufacturer’s guidelines.

2. To begin with, carry out a baseline correction with an empty sample compartment.

3. Check that the reference plate is correctly positioned in the light path by first measuring the windows without glass (usually columns 1 and 2). The label showing the reference plate type must be visible from above.

4. Check that the device’s display has not changed. In microplates with very large beams, the measurement beam may touch the window frame. If this is the case, you will notice a change in the device’s display.
   - If necessary, adjust the position of the reference plate holder until the light beam shines through the empty window unimpeded.
   - The reference plate is correctly positioned if the display values from the zero adjustment performed in step 2 (baseline correction) do not change.

5. Carry out the filter measurement in a closed sample compartment as carefully as you would carry out a sample measurement (open sample compartments produce incorrect results).

A careful handling of the reference plates is very important. An impurity on the glass surfaces, leads to wrong measurement results.
MEASUREMENT PARAMETERS FOR CHECKING PHOTOMETRIC ACCURACY

Generally speaking, reference plates can also be measured using a slit width that differs from the information provided on the calibration certificate. However, please note that using large slit widths may result in slight deviations from the values stated on the calibration certificate. In cases of doubt, it is therefore advisable to choose as small a slit width as possible. We recommend taking several measurements and then using the mean value to avoid errors during evaluation.

MEASUREMENT PARAMETERS FOR CHECKING WAVELENGTH ACCURACY

Ensure that you have selected the correct measurement parameters before scanning the absorbance curve to detect peak positions. Incorrect parameters may distort the absorbance curve and thus shift the actual positions of peaks. Please use the settings stated on the accompanying calibration certificate. It should be noted that changing the slit width of the microplate reader can cause the absorbance maxima to shift slightly. Ignore any influence that the spectral bandwidth from 1 nm to 2 nm has on peak positions. Peak heights, however, may vary greatly following changes to the slit width due to their narrow nature. As a result, filters for checking wavelength accuracy are usually unsuitable for checking absorbance accuracy.

INTERPRETING MEASUREMENT RESULTS WITH REFERENCE PLATES FOR CHECKING PHOTOMETRIC AND WAVELENGTH ACCURACY

The measurement uncertainties stated on the calibration certificate only refer to measurements conducted by Hellma Analytics and apply solely to the measurement conditions at the company (spectrophotometer used, environmental influences such as temperature, air humidity, user influence, and reference materials used). The smallest possible measurement uncertainty that can be achieved by the user can then be derived by statistically combining the measurement uncertainty stated on the calibration certificate with all the user’s uncertainty contributions, such as the wavelength scale tolerance of the microplate reader used and other influences on measurement accuracy (environmental factors such as temperature, air humidity, user influence, etc.). For further literature on correctly calculating measurement uncertainty, please refer to chapter 8 of this user manual.
Continuously assured quality: 
Recertification intervals for reference materials

As 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 9001:2008 “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.

Important parameters for recertification

The length of intervals between the recertification of reference materials depends on how frequently materials are used, the wear associated with this, accuracy requirements, and the requirements of a company’s internal auditing. In general, a recertification interval of 12 months is recommended for checking and recertifying glass filters during the first two years of use, with an interval of 24 months thereafter. We recommend verifying and recertifying liquid filters within a maximum of 12 months. Intervals should be specified individually in accordance with your QM system.

Fast and reliable – recertification service

In our DAkkS-accredited calibration laboratory, your reference materials are cleaned and are recertified in accordance with your requirements using a high-performance spectrophotometer. If necessary, filters are repaired, or are exchanged following a consultation. You will receive your filters with a new DAkkS calibration certificate or Hellma Analytics calibration certificate.

Filters are usually recertified within five working days of their arrival at the calibration laboratory.

Recertification of reference materials from other manufacturers.

We also recertify reference materials for UV/Vis spectroscopy from other suppliers. If you require a quotation first, please send your inquiry via email to your local Hellma partner.

Returning your reference materials for recertification

Efficient processing of the reference materials you send us ensures that you will be able to use your filters again within just a few days. We need your support to make this possible. Please include all information needed to process the reference materials:

- Article no.*
- Serial no.*
- Wavelength(s) to be measured*
- Slit width(s) to be measured*
- Documentation of measurement data prior to cleaning** Yes / No
- Quotation no. (if you have already received a quotation from us)
- Billing address
- Delivery address (if different from billing address)
- Special requests, e.g. additional wavelengths etc

Please enclose a copy of your order or send this via email to your local Hellma partner.

If you send your reference materials with only a delivery note, it is essential that you indicate your order number. Please include this on the delivery note, otherwise we will be unable to process your order.

GLASS FILTERS  
RECERTIFICATION EVERY 24 MONTHS

PURCHASE 1st YEAR 2nd YEAR 4th YEAR 6th YEAR 8th YEAR

●

●

●

●

●

LIQUID FILTERS  
RECERTIFICATION INTERVAL OF 12 MONTHS IS RECOMMENDED

PURCHASE 1st YEAR 2nd YEAR 3rd YEAR 4th YEAR 5th YEAR 6th YEAR 7th YEAR 8th YEAR

●

●

●

●

●

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●

●

Please note:

Liquid filters can only be sent when the external temperature is above 4°C, as the liquid can freeze, which will destroy the reference materials.

Please include this on the delivery note, otherwise we will be unable to process your order.

* This information is not necessary if you enclose a copy of the current calibration certificate.
** Documentation of measurement data prior to cleaning, please note this on your order. Depending on your quality management requirements, you have two options:
1. Documentation of measurement data prior to cleaning with DAkkS certificate.
2. Documentation of measurement data prior to cleaning with simple measurement report.
6. FAQ

6.1 How does the recertification of my filters work? Hints for the return and recertification of your reference materials can be found on page 55.

6.2 Why do holmium glass filters become cloudy? Will this interfere with the measurement? The glass material used for this filter is somewhat hygroscopic, which means that the filters become coated with a kind of water film. The film does not interfere with measure- ments or change the characteristic peak positions of holmium. The filter can be easily wiped down using alcohol and a soft cloth. The filter should generally be stored in a dry place.

6.3 How long can a calibration standard be used for in total? Depending on the conditions in which they are used and stored, as well as how they are maintained, filters usually last for many years. We recommend having filters regularly recertified so that any signs of deterioration can be recognized at an early stage.

6.4 How often should filters be recertified? Certified reference materials should be recertified at regu- lar intervals to check that the values stated on the calibra- tion certificate are still valid. It is up to the user to decide on the regularity of these intervals, which should take into account the use, storage and usage conditions of the filter in the laboratory. To establish a statistical database for de- termining recertification intervals, we recommend having all reference materials recertified at least every 12 months during their first two years of use, and then selecting a sui- table recertification interval based on the values measured. (See also chapter 5).

6.5 What do the tolerances on the calibration certificate tell us and how can they be correctly interpreted? The measurement uncertainties that appear on calibration certificates only refer to measurements conducted by Hellma Analytics and apply solely to the measurement con- ditions at the company (spectrophotometer used, environ- mental influences such as temperature, air humidity, user influence, reference materials used, etc.). Consequently, the measurement uncertainties of the NIST reference materials used to ensure traceability have been mathematically com- bined with the measurement uncertainty statistics calcula- ted by Hellma Analytics. The value provided is therefore an expanded measurement uncertainty (double standard devia- tion, coverage factor k=2). This means that the actual value is 95% certain to fall within this range. To correctly calcula- te the measurement uncertainties valid for their measuring system, reference material users should follow the same steps, mathematically/statistically combining the measure- ment uncertainties provided with the measurement uncer- tainty statistics they have calculated themselves for a par- ticular spectrophotometer and relevant conditions (see ISO/ IEC Guide 98-3:2008 ‘Guide to the Expression of Uncertainty in Measurement’).

6.6 What is a baseline correction? Baseline corrections are carried out with an empty cuvette holder to compensate for the lamps. Since lamps emit light at different strengths at various wavelengths, baseline cor- rections (also known as zero auto) are carried out to determi- ne a zero value. Baseline corrections are usually performed automatically when the spectrophotometer is started up, but can also be carried out manually.

6.7 What is background correction? Background correction is carried out to eliminate any influ- ences that extend beyond the sample’s properties. In double beam photometers, background correction is performed by simultaneously measuring the comparison cuvette in the reference beam path. This comparison cuvette usually con- tains pure solvent. In single beam photometers, background correction is carried out before the actual sample measure- ment is taken by measuring the comparison cuvette. The va- lues obtained for the comparison cuvette are then deducted from the values of the sample measurement.

6.8 Why does the calibration certificate for the filter set used to determine spectral resolution look different to other calibration certificates? Determining spectral resolution does not fall within our scope of accreditation. The filter set for determining spectral resolution therefore cannot be issued with a D44K5 calibration certificate or calibration mark. That is why this calibration certificate looks dif- ferent from other calibration certificates for filter sets.

6.9 Why does Hellma Analytics no longer offer Potassium dichromate filters for checking photometric accuracy with sulfuric acid as a solvent, as described in the European Pharmacopoeia? In the past, certified reference materials for checking pho- tometric accuracy in the UV range contained a solution of Potassium dichromate in sulfuric acid and were manufac- tured in strict compliance with European Pharmacopoeia requirements. Over a number of years, Hellma Analytics no- ticed a continuous decrease in the absorbance values of the ‘Potassium dichromate dissolved in sulfuric acid’ filter during daily calibrations. We do not have a sufficient explanation for why this happens, but we assume that the comparatively high ionic strength of sulfuric acid causes mixed chromium (VI) complexes to form. To compensate for this behavior, over which we have no control, filters would need to be recertified much more regularly. Another possibility would be preparing new solutions every time the spectrophotometer is chok- ked. As a simple alternative, we offer a liquid filter that uses ‘Potassium dichromate dissolved in perchloric acid’. This type of liquid filter for checking photometric accuracy has proven itself as a reliable and very stable standard for many years. No changes in absorbance properties comparable to those of the sulfuric acid model are known for this filter. Hellma Analytics cuvettes are permanently sealed, eliminating con- cerns about the toxicity of perchloric acid. Furthermore, the European Pharmacopoeia states that ‘suitable certified re- ference materials’ may also be used, which undoubtedly ap- plies to our perchloric acid solvent model. This model also contains a formulation described by NIST.

6.10 Why does the weight of Potassium dichromate filters seem to change after every recertification? Due to measurement uncertainties, measurement values may fall within a specific range. This leads to an apparent change in weight from qualification to qualification, as the initial weight is calculated directly from the measured ab- sorbance values. Earlier versions of regulatory codes stipu- lated that filters for checking photometric accuracy had to contain 60.6 mg/Kg Potassium dichromate, and allowed a to- lerance of 0.01 Abs. More current versions of the European Pharmacopoeia have replaced this very strict provision, now accepting weights between 57.0 mg/l and 63.0 mg/l. The spe- cific absorbance calculated (see European Pharmacopoeia, chapter 2.2.25) is now stated with a margin of tolerance.

6.11 Why are these peaks measured for certifying holmium glass and didymium glass filters? Measurement errors are low in medium to high transmit- tance ranges. As a result, peaks in the range from 0 Abs to 1.0 Abs (corresponds to 100% - T to 10% T) are preferred for certification.

6.12 How do I calculate my measurement uncertainty? The measurement uncertainties stated on the calibration cer- tificate only refer to measurements conducted by Hellma Analytics and apply solely to the measurement conditions at the company (spectrophotometer used, environmental influences such as temperature, air humidity, user influence, reference materials used, etc.). The smallest possible measurement un- certainty that can be achieved by the user can then be derived by statistically combining the measurement uncertainty stated on the calibration certificate with all the user’s uncertainty contribu- tions, such as the wavelength scale tolerance of the spectropho- tometer used and other influences on measurement accuracy (environmental factors such as temperature, air humidity, user influence, etc.).

**Example of calculating standard measurement uncertainty for a neutral density glass filter (highly simplified): The calibration certificate states the following measurement values and measurement uncertainties:**

<table>
<thead>
<tr>
<th>Value</th>
<th>Measurement Value</th>
<th>Measurement Uncertainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEASURED VALUE</td>
<td>0.2562</td>
<td>+/- 0.0024 Abs</td>
</tr>
<tr>
<td>expanded measurement uncertainty</td>
<td>+/- 0.0024 Abs</td>
<td></td>
</tr>
</tbody>
</table>

Next, you must calculate the measurement error specific to your spectrophotometer (xb) – refer to the operating instructions for more details – and define a value for the measuring error due to environmental influences at your company (xu) [with “tempera- ture and air humidity”].

**Example of measuring error parameters:**

<table>
<thead>
<tr>
<th>Specophotometer (xb)</th>
<th>+/- 0.001 Abs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental influences (xu)</td>
<td>+/- 0.001 Abs</td>
</tr>
</tbody>
</table>

Calculating standard measurement uncertainty (MJ):

\[
MJ = xb + xu = 0.001 + 0.001 = 0.002 Abs
\]

Expanded measurement uncertainty is calculated by multiplying this value by coverage factor k.

As shown here, in practice it is often easier to simply add up uncertainty contributions than to combine them statistically. However, the method used to determine measurement uncer- tainty depends on the specifications of your quality system and your measurement accuracy requirements. For further litera- ture on correctly calculating measurement uncertainty, please refer to the recommendations for further reading in chapter 8 of this user manual.

6.13 Control chart? See page 41
7. GLOSSARY

Abbreviations:

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>absorbance</td>
</tr>
<tr>
<td>ASTM</td>
<td>American Society for Testing and Materials</td>
</tr>
<tr>
<td>BG</td>
<td>Specific term for Schott glass</td>
</tr>
<tr>
<td>DAB</td>
<td>Deutsches Arzneibuch (German Pharmacopoeia)</td>
</tr>
<tr>
<td>DAAKs</td>
<td>Deutsche Akkreditierungsvorstand (National accreditation body for the Federal Republic of Germany)</td>
</tr>
<tr>
<td>DAR</td>
<td>Deutscher Akkreditierungsrat (German accreditation body)</td>
</tr>
<tr>
<td>DKD</td>
<td>Deutscher Kalibrierdienst (German calibration body)</td>
</tr>
<tr>
<td>Ph. Eur.</td>
<td>European Pharmacopoeia</td>
</tr>
<tr>
<td>FAQs</td>
<td>Frequently asked questions</td>
</tr>
<tr>
<td>GLP</td>
<td>Good laboratory practice</td>
</tr>
<tr>
<td>GMP</td>
<td>Good manufacturing practice</td>
</tr>
<tr>
<td>I</td>
<td>Intensity of light beam</td>
</tr>
<tr>
<td>IO</td>
<td>Original intensity of light beam</td>
</tr>
<tr>
<td>k</td>
<td>Coverage factor for measurement uncertainty</td>
</tr>
<tr>
<td>λmax</td>
<td>Maximum peak at defined wavelength</td>
</tr>
<tr>
<td>λmin</td>
<td>Minimum peak at defined wavelength</td>
</tr>
<tr>
<td>NG</td>
<td>Neutral density glass</td>
</tr>
<tr>
<td>NIR</td>
<td>Near-infrared</td>
</tr>
<tr>
<td>NIST</td>
<td>National Institute of Standards and Technology</td>
</tr>
<tr>
<td>PTB</td>
<td>Physikalisch-Technische Bundesanstalt (Germany’s national metrology institute)</td>
</tr>
<tr>
<td>SRM®</td>
<td>Standard Reference Material (registered trademark of NIST)</td>
</tr>
<tr>
<td>USP</td>
<td>United States Pharmacopeia</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet (wavelength range 200 – 380 nm)</td>
</tr>
<tr>
<td>Vis</td>
<td>Visible (visible wavelength range 380 – 780 nm)</td>
</tr>
</tbody>
</table>

Absorbance (Abs): When light falls on or passes through a sample, the quantity of absorbed light is equal to the difference between the original intensity I0 and the intensity I after interaction with the sample. This is because part of the irradiated light is transferred to the molecules, causing the beam to have a smaller output when it exits the sample. The extent to which light is absorbed is determined by the principles of the Beer-Lambert law. The amount of absorbed light can be expressed as transmittance (see definition) or absorbance. Absorbance is defined as: Abs = -log T. According to the relevant standard, this parameter is referred to as spectral optical density on transmittance (‘optical density’).

Optical density: see absorbance

Visible range: Part of the optical spectrum that stretches from 380 nm to 780 nm of the wavelength range of electromagnetic radiation. This range is generally referred to as light. This is the only range in which the human eye can ‘see’ electromagnetic radiation.

Spectral resolution: This refers to a measuring system’s ability to separate individual wavelength ranges.

Spectral bandwidth: Wavelength range that appears with a continuum at the exit slit when the monochromator is exposed to irradiation. Spectral bandwidth is determined by the bandwidth of emitted radiation where the light has reached half the maximum intensity.

Spectral optical density on transmittance: see absorbance

Transmittance (T): When light falls on or passes through a sample, the quantity of absorbed light is equal to the difference between the original intensity I0 and the intensity I after interaction with the sample. This is because part of the irradiated light is transferred to the molecules, causing the beam to have a smaller output when it exits the sample. The extent to which light is absorbed is determined by the principles of the Beer-Lambert law. The amount of absorbed light can be expressed as transmittance (see definition) or absorbance. Transmittance is normally expressed as a fraction of 1 or as a percentage, and is defined as follows: T = I/I0 or %T = (I/I0) * 100.

Ultraviolet range (UV range): Also known as UV radiation, this is the short-wave part of the optical radiation spectrum. UV radiation has a wavelength range of 200 nm to 380 nm.

Wavelength: Wavelength is the distance between two identical, adjacent corresponding points of the same wave phase at a certain point in time.

8. LITERATURE REFERENCES

- Standards and Best Practice in Absorption Spectrometry; Edited by C. Burgess and T. Frost UVSG, ISBN 0-632-05313-5 Blackwell Service
- Qualitätssicherung in der Analytischen Chemie; Werner Funk, Vera Dammann, Gerhild Donnewert, ISBN 10: 3-527-31112-2, Verlag WILEY-VCH
- European Pharmacopoeia (Ph.Eur.)
- DKD3
- United States Pharmacopeia (USP)
# 9. PRODUCT OVERVIEW

## LIQUID FILTERS WITH DAKKS CERTIFICATE

<table>
<thead>
<tr>
<th>TYPE</th>
<th>MATERIAL</th>
<th>WAVELENGTH (nm)</th>
<th>ARTICLE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glass Filters for testing the wavelength accuracy</td>
<td>Holmium Glass Filter F1</td>
<td>279, 361, 453, 564, 638</td>
<td>666-F1-339</td>
</tr>
<tr>
<td></td>
<td>Didymium Glass Filter F7W</td>
<td>329, 472, 512, 481, 875</td>
<td>666-F7W-323</td>
</tr>
<tr>
<td>Glass Filters for testing the photometric accuracy</td>
<td>Neutral Density Glass Filter F390: 0.06 Abs</td>
<td>666-F390-25</td>
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<td>Neutral Density Glass Filter F2: 0.25 Abs</td>
<td>666-F2-25</td>
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<tr>
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<td>Neutral Density Glass Filter F1: 0.3 Abs</td>
<td>666-F1-3</td>
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</tr>
<tr>
<td></td>
<td>Neutral Density Glass Filter F20: 1.8 Abs</td>
<td>666-F20-36</td>
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</tr>
<tr>
<td></td>
<td>Neutral Density Glass Filter F20, F15 Abs</td>
<td>666-F20-36</td>
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</tr>
<tr>
<td></td>
<td>Neutral Density Glass Filter F301: 2.5 Abs</td>
<td>666-F301-361</td>
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</tr>
<tr>
<td></td>
<td>Neutral Density Glass Filter F303: 3.0 Abs</td>
<td>666-F303-361</td>
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</tr>
<tr>
<td></td>
<td>Didymium Glass Filter F7A: ca. 0.5 – 1.0 Abs</td>
<td>666-F7A-323</td>
<td></td>
</tr>
<tr>
<td>Glass Filter for testing the photometric accuracy and wavelength accuracy</td>
<td>Didymium Glass Filter F7</td>
<td>A: 270, 280, 297, 321, 362</td>
<td>666-F7-323</td>
</tr>
<tr>
<td>Empty filter mount</td>
<td>Reference Filter frame made of aluminum (without glass)</td>
<td>666-F0-71</td>
<td></td>
</tr>
<tr>
<td>Sets for testing the photometric accuracy and wavelength accuracy</td>
<td>Complete Glass Filter Set: F1, F2, F3, F4, F4 (Abs: 0.25, 0.5, 1.0)</td>
<td>A: 460, 665, 664, 1, 590, 635 W: 279, 361, 453, 564, 638</td>
<td>666S500</td>
</tr>
<tr>
<td></td>
<td>Glass Filter Set: F3, F4, F7 (Abs: 0.5, 1.0, 7): ca. 0.5 – 1.0</td>
<td>A (F7): 270, 280, 297, 321, 362</td>
<td>666S501</td>
</tr>
<tr>
<td></td>
<td>Glass Filter Set: F2, F3, F4 (Abs: 0.25, 0.5, 1.0)</td>
<td>A: 460, 665, 664, 1, 590, 635</td>
<td>666S502</td>
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<tr>
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<td>Glass Filter Set: F1, F2, F3, F4, F7, F15 (Abs: 0.25, 0.5, 1.0, F7: ca. 0.5 – 1.0)</td>
<td>A (F7): 270, 280, 297, 321, 362</td>
<td>666S503</td>
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<tr>
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<td>Glass Filter Set: F20, F301, F303, F30 (Abs: 0.3, 1.5, 2.4)</td>
<td>A: 460, 665, 664, 1, 590, 635 W: 279, 361, 453, 564, 638</td>
<td>666S504</td>
</tr>
<tr>
<td></td>
<td>Glass Filter Set: F1, F2, F3, F4, F5 (Abs: 0.5, 1.0)</td>
<td>A: 460, 665, 664, 1, 590, 635</td>
<td>666S505</td>
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<tr>
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<td>Glass Filter Set: F2, F3, F4, F5 (Abs: 0.25, 0.5, 1.0)</td>
<td>A: 460, 665, 664, 1, 590, 635</td>
<td>666S506</td>
</tr>
<tr>
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<td>Glass Filter Set: F30, F301, F303 (Abs: 0.04, 2.5, 3.4)</td>
<td>A: 460, 645, 656, 1, 595, 635</td>
<td>666S300</td>
</tr>
</tbody>
</table>

A: Wavelength for absorbance  W: Wavelength for wavelength accuracy

## LIQUID FILTERS WITH DAKKS CERTIFICATE

<table>
<thead>
<tr>
<th>TYPE</th>
<th>MATERIAL</th>
<th>WAVELENGTH (nm)</th>
<th>ARTICLE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid Filters for testing the photometric accuracy</td>
<td>29 mg/l potassium dichromate in HClO4</td>
<td>667-UV020</td>
<td></td>
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<tr>
<td></td>
<td>40 mg/l potassium dichromate in HClO4</td>
<td>667-UV040</td>
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<tr>
<td></td>
<td>40 mg/l potassium dichromate in HClO4</td>
<td>667-UV060</td>
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<td>80 mg/l potassium dichromate in HClO4</td>
<td>667-UV080</td>
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<td>140 mg/l potassium dichromate in HClO4</td>
<td>667-UV110</td>
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<td>160 mg/l potassium dichromate in HClO4</td>
<td>667-UV160</td>
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<td>200 mg/l potassium dichromate in HClO4</td>
<td>667-UV200</td>
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<td></td>
<td>400 mg/l potassium dichromate in HClO4</td>
<td>667-UV400</td>
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<tr>
<td></td>
<td>Parachloric acid (Reference filter)</td>
<td>667-UV14</td>
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</tr>
<tr>
<td>Filter Set for UV-range: 241, 287, 361, 536, 640</td>
<td>667-UV001</td>
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<tr>
<td>Filter Set for UV400-range: 241, 287, 361, 536, 640</td>
<td>667-UV51</td>
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</tr>
<tr>
<td>Filter Set for UV-51, UV52, UV53, UV54, UV59</td>
<td>667-UV25</td>
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<td></td>
</tr>
<tr>
<td>Filter Set for UV-51, UV52, UV53, UV54, UV59</td>
<td>667-UV35</td>
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</tr>
<tr>
<td>Filter Set for Nitric acid (0.5 Abs)</td>
<td>667-UV35</td>
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</tr>
<tr>
<td>Filter Set for Nitric acid (0.5 Abs)</td>
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<tr>
<td>Filter Set for Nitric acid (0.5 Abs)</td>
<td>667-UV35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*with Hellma Analytics calibration certificate*
### 9. PRODUCT OVERVIEW

#### LIQUID FILTERS WITH DAKKS CERTIFICATE

<table>
<thead>
<tr>
<th>TYPE</th>
<th>MATERIAL</th>
<th>WAVELENGTH (nm)</th>
<th>ARTICLE NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid Filters for testing the wavelength accuracy acc. to USP &lt;857&gt;</td>
<td>Holmium in perchloric acid</td>
<td>241, 250, 278, 335, 361, 385, 416, 452, 468, 495, 536, 640</td>
<td>667001USP</td>
</tr>
<tr>
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<td>Sodium dichromate filters: UV60/0/UV14</td>
<td>(Abs: 0.3 – 0.9)</td>
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</tr>
<tr>
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<td>Sodium nitrite in H2O: UV11/11H</td>
<td>385 (cut-off), 416; 452; 468; 485; 536; 640</td>
<td>667003</td>
</tr>
<tr>
<td></td>
<td>Neutral density glass filters: F2, F3, F4</td>
<td>440; 465; 546.1; 590; 635</td>
<td>667004</td>
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<tr>
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<td>Potassium dichromate filters: UV60/0/UV14</td>
<td>(Abs: 0.25; 0.5; 1.0)</td>
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<tr>
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<td>Sodium nitrite in H2O: UV11/11H</td>
<td>385 (cut-off), 416; 452; 468; 485; 536; 640</td>
<td>667006</td>
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<td>Potassium dichromate 60mg/L: UV60/14</td>
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<td>Neutral density glass filters: F2, F3, F4</td>
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<td>Potassium dichromate filters: UV60/14</td>
<td>(Abs: 0.3 – 0.9)</td>
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<td>Sodium nitrite in H2O: UV11/11H</td>
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<td>Potassium dichromate filters: UV60/14</td>
<td>(Abs: 0.25; 0.5; 1.0)</td>
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<td>Sodium nitrite in H2O: UV11/11H</td>
<td>385 (cut-off), 416; 452; 468; 485; 536; 640</td>
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<td>Neutral density glass filters: F2, F3, F4</td>
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<td>Potassium dichromate filters: UV60/14</td>
<td>(Abs: 0.3 – 0.9)</td>
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<td>Sodium nitrite in H2O: UV11/11H</td>
<td>385 (cut-off), 416; 452; 468; 485; 536; 640</td>
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<td>Neutral density glass filters: F2, F3, F4</td>
<td>440; 465; 546.1; 590; 635</td>
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<td>Potassium dichromate filters: UV60/14</td>
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<td>Neutral density glass filters: F2, F3, F4</td>
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<td>Potassium dichromate filters: UV60/14</td>
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<td>385 (cut-off), 416; 452; 468; 485; 536; 640</td>
<td>667022</td>
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#### FILTER SETS ACC. TO PH.EUR AND USP <857> WITH DAKKS CERTIFICATE

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<thead>
<tr>
<th>TYPE</th>
<th>CONTENT</th>
<th>WAVELENGTH (nm)</th>
<th>ARTICLE NO.</th>
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<tbody>
<tr>
<td>Complete Filter Set for testing the spectrophotometer according to Ph.Eur.</td>
<td>Potassium dichromate filters: UV60/0/UV14</td>
<td>(Abs: 0.3 – 0.9)</td>
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<td>Sodium nitrite in H2O: UV11/11H</td>
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<td>Potassium dichromate filters: UV60/14</td>
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<td>Sodium nitrite in H2O: UV11/11H</td>
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<td>Potassium dichromate filters: UV60/0/UV14</td>
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<td>Sodium nitrite in H2O: UV11/11H</td>
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<td>Potassium dichromate filters: UV60/14</td>
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<td>Sodium nitrite in H2O: UV11/11H</td>
<td>385 (cut-off), 416; 452; 468; 485; 536; 640</td>
<td>667008</td>
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<td></td>
<td>Potassium dichromate filters: UV60/14</td>
<td>(Abs: 0.3 – 0.9)</td>
<td>667009</td>
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<td>Sodium nitrite in H2O: UV11/11H</td>
<td>385 (cut-off), 416; 452; 468; 485; 536; 640</td>
<td>667010</td>
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<td>Potassium dichromate filters: UV60/14</td>
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<td>Sodium nitrite in H2O: UV11/11H</td>
<td>385 (cut-off), 416; 452; 468; 485; 536; 640</td>
<td>667012</td>
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<td>Potassium dichromate filters: UV60/14</td>
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<td>Sodium nitrite in H2O: UV11/11H</td>
<td>385 (cut-off), 416; 452; 468; 485; 536; 640</td>
<td>667014</td>
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#### REFERENCE PLATES FOR QUALIFYING MICROPLATE READERS WITH DAKKS CERTIFICATE

With reference plates from Hellma Analytics you can check the photometric and wavelength accuracy of microplate readers. They have the same dimensions as a microplate with 96 wells and a 6.6 mm diameter per window (height 14.5 x width 125 x length 85.5 mm).

<table>
<thead>
<tr>
<th>TYPE</th>
<th>USAGE</th>
<th>MATERIAL</th>
<th>WAVELENGTH (nm)</th>
<th>ARTICLE NO.</th>
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<tbody>
<tr>
<td>666-R013</td>
<td>To check photometric accuracy</td>
<td>Neutral density glass Filter</td>
<td>405; 450; 490; 650</td>
<td>666R013</td>
</tr>
<tr>
<td>666-R113</td>
<td>To check photometric accuracy</td>
<td>Neutral density glass Filter</td>
<td>NG 1; 1.5; 2; 2.5</td>
<td>666R113</td>
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<tr>
<td>666-R013</td>
<td>To check photometric accuracy</td>
<td>Neutral density glass Filter</td>
<td>405; 450; 490; 650</td>
<td>666R013</td>
</tr>
<tr>
<td>666-R113</td>
<td>To check photometric accuracy</td>
<td>Neutral density glass Filter</td>
<td>NG 1; 1.5; 2; 2.5</td>
<td>666R113</td>
</tr>
</tbody>
</table>