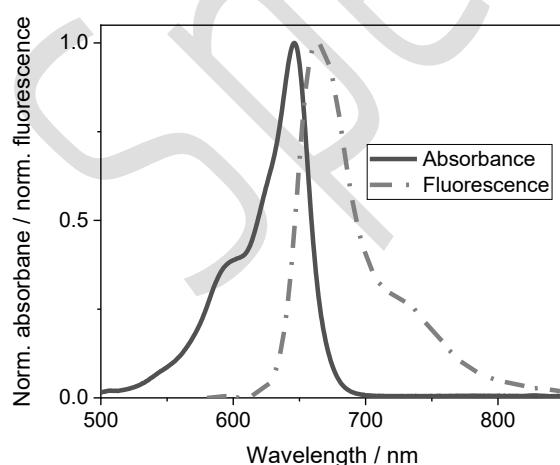


## Certified Reference Material (CRM)

### BAM-F021

#### Fluorescence Quantum Yield Standard

Measurand <sup>a)</sup>	Certified value $\Phi_f$ <sup>b)</sup>	Uncertainty $U$ <sup>c)</sup>
BAM-F021	0.151	0.010
<p>a) Ethanolic dye solution of BAM-F021, which shows an absorbance at the excitation wavelength <math>A(\lambda_{ex})</math> of 0.105.</p> <p>b) BAM-F021 dissolved in absolute ethanol is certified for use as fluorescence quantum yield standard for the relative determination of the fluorescence quantum yield (<math>\Phi_f</math>) and for controlling and validating the performance of integrating sphere-based fluorescence instruments for the absolute determination of <math>\Phi_f</math> at <math>T = (22 \pm 2) \text{ }^\circ\text{C}</math>.</p> <p>c) Estimated expanded uncertainty <math>U</math> with a coverage factor of <math>k = 2</math> corresponding to a level of confidence of approx. 95 %, as defined in the Guide to the expression of uncertainty.<sup>1</sup></p>		



**Fig. 1** Normalized absorbance and fluorescence spectra of the ethanolic solution of BAM-F021 used for certification.

#### End of Validity

This certificate is valid for 2 years after dispatch, if the solid CRM is stored at 2 - 8 °C in the dark. BAM-F021 can be shipped at room temperature.

**Sample No.:**

**Date of dispatch:**

## Material Description

The fluorescence quantum yield standard BAM-F021 is supplied as a solid in a tightly closed glass bottle<sup>x)</sup>. The Standard Operation Procedure (SOP) based on the publication of C. Wuerth et al. in Nature Protocols 2013<sup>2</sup> that describes the correct use of CRM BAM-F021 is included. An open access version of the revised and finally submitted manuscript and supporting information approved by Nature Protocols can be found under <https://nbn-resolving.org/urn:nbn:de:kobv:b43-294202>.

<sup>x)</sup>The bottle containing BAM-F021 and the box used as package have labels with different BAM logos as BAM meanwhile changed its logo.

## Recommended Use

BAM-F021 dissolved in absolute ethanol is certified for use as a  $\Phi_f$  standard for the relative determination of the fluorescence quantum yields of transparent luminophore solutions using excitation wavelengths between 575 nm and 650 nm (excitation within the dye's longest wavelength absorption band). BAM-F021 can be also employed for controlling and validating the performance of integrating sphere-based fluorescence instruments used for the absolute determination of  $\Phi_f$ .

## Handling

Please follow the instructions given in the Standard Operation Procedure (SOP) provided with the material.

Prerequisites for the reliable relative determination of  $\Phi_f$  with BAM-F021 as reference standard is the use of calibrated spectrometers to determine the number of absorbed and emitted photons.<sup>3-4</sup>

Recommended instrument calibrations and characterizations include:

- a.) Calibration of the wavelength and intensity scale of the absorption spectrometer,
- b.) Calibration of the wavelength scale of the spectrofluorometer,
- c.) Determination of the range of linearity of the detectors of the spectrometers,
- d.) Determination of the wavelength-dependent relative spectral responsivity of the spectrofluorometer's detection channel and consideration of the photonic nature of the emitted light.

For the preparation of a dye stock solution, the solid dye should be dissolved in 10 mL absolute ethanol (spectrophotometric grade  $\geq 99.8$  % (GC)). Use of a freshly opened bottle of ethanol is recommended to guarantee a minimum water content.

For the relative determination of  $\Phi_f$  in a  $0^\circ / 90^\circ$  measurement geometry of the spectrofluorometer, an absorbance between 0.02 – 0.05 at the selected excitation

wavelength is recommended to minimize reabsorption effects.<sup>2</sup> For the absolute determination of  $\Phi_f$  with integrating sphere-based fluorescence instruments, the choice of the optimum absorbance where reabsorption effects are minimum, depends on the size of the integrating sphere and the wavelength-dependent reflectivity of the sphere coating.<sup>3</sup> The recommended measurement conditions are summarized in **Table 1**.

**Table 1** Recommended measurement conditions.

Measurement condition	
Excitation wavelength ( $\lambda_{ex}$ )	575 nm - 650 nm
Absorbance at $\lambda_{ex}$	0.02 - 0.05 (relative measurement) 0.03 - 0.15 (absolute measurement)
Temperature	20 - 25 °C

For further information, see the SOP provided by BAM and the comprehensive description of the procedures for the absolute and relative determination of transparent luminophore samples given in Nature Protocols.<sup>2</sup> An open access version of the revised and finally submitted manuscript and supporting information approved by Nature Protocols can be found under <https://nbn-resolving.org/urn:nbn:de:kobv:b43-294202>.

### Transport and Storage

BAM-F021 should be shipped at room temperature. After receipt, the CRM must be stored in a refrigerator at 2 - 8 °C. Under these conditions, it will preserve its certified properties for **24 months after delivery**.

When preparing the measurement solution, care must be taken to avoid moisture uptake.

The ethanolic solution of BAM-F021 must be stored in the dark at 2 - 8 °C in a tightly closed container to prevent water uptake. Stock solutions of this dye with typical absorbances at the absorbance maximum of 2 can be stored under these conditions for 6 months in the dark at 2 - 8 °C. Dilute solutions with an absorbance at the maximum of 0.3 or less should be disposed after use.

BAM is not responsible for changes in the certified properties that occur during storage of the material at the customer's premises, especially not in the case of opened vials and prepared dye solutions.

### Analytical Method used for Certification

The fluorescence quantum yield  $\Phi_f$  is a direct measure for the efficiency of the conversion of absorbed light into emitted light.  $\Phi_f$  is defined as the quotient of the number of emitted photons ( $N_{em}(\lambda)$ ) and the number of absorbed photons ( $N_{abs}(\lambda)$ ).

The absolute determination of the fluorescence quantum yield was performed with a calibrated custom-built integrating sphere setup at  $T = (22 \pm 2) \text{ }^\circ\text{C}$ . The measurement conditions used are given in Table 2.

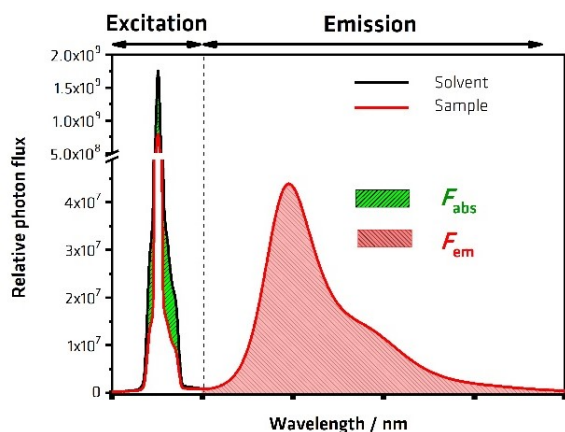
**Table 2** Measurement conditions used for certification: Excitation wavelength  $\lambda_{ex}$ , absorbance at the excitation wavelength  $A(\lambda_{ex})$ , wavelength of the maximum absorbance  $\lambda_{max}$ , maximum absorbance  $A^s(\lambda_{max})$ , and concentration  $c^s$  of a selected dye solution used.

CRM	$\lambda_{ex}/ \text{nm}$	$A(\lambda_{ex})$	$\lambda_{max}/ \text{nm}$	$A^s(\lambda_{max})$	$c^s/ (\text{mol/L})$
BAM-F021	600	0.105	646	0.271	8.2E-06

With such a setup, the fluorescence quantum yield  $\Phi_f$  can be directly measured as the quotient of the relative photon flux emitted from the sample ( $F_{em}$ ) and the relative photon flux absorbed from the incident light at the chosen excitation wavelength ( $F_{abs}$ ), see equation [1].

$$\Phi_f = \frac{F_{em}}{F_{abs}} \quad [1]$$

The relative absorbed photon flux  $F_{abs}$  and the relative emitted photon flux  $F_{em}$  are calculated by separating the measured spectra of sample and blank in an excitation and an emission region as shown in Figure 2. Subsequently, i)  $F_{abs}$  is calculated from the integrated difference of the spectrally corrected signals ( $I_c$ ) of the blank (the solvent used for dissolving the dye) and the sample in the wavelength region of excitation (see equation [2]); ii.)  $F_{em}$  is determined from the integrated difference of the spectrally corrected signals of the sample and the blank in the spectral region of the emission (see equation [3]). Measurements with a blank are indicated with the index b, measurements with the sample with the index x, respectively. The emission correction curve (relative spectral responsivity  $s(\lambda_{em})$ , see equation [4]) was determined in reference to the spectral radiance and converted to the relative spectral photon flux ( $q$ ) (see equation [2] and equation [3]).



**Fig. 2** Scheme of the determination of the relative photon flux emitted from the sample (red area) and the relative absorbed photon flux (green area) measured with an integrating sphere setup.

$$F_{\text{abs}} = \int_{\lambda_{\text{ex1}}}^{\lambda_{\text{ex2}}} (q_{\text{b}}(\lambda_{\text{ex}}) - q_{\text{x}}(\lambda_{\text{ex}})) d\lambda_{\text{ex}} \quad [2]$$

$$= \int_{\lambda_{\text{ex1}}}^{\lambda_{\text{ex2}}} (I_{\text{c,b}}(\lambda_{\text{ex}}) - I_{\text{c,x}}(\lambda_{\text{ex}})) \lambda_{\text{ex}} d\lambda_{\text{ex}}$$

$$F_{\text{em}} = \int_{\lambda_{\text{em1}}}^{\lambda_{\text{em2}}} (q_{\text{x}}(\lambda_{\text{em}}) - q_{\text{b}}(\lambda_{\text{em}})) d\lambda_{\text{em}} \quad [3]$$

$$= \int_{\lambda_{\text{em1}}}^{\lambda_{\text{em2}}} (I_{\text{c,x}}(\lambda_{\text{em}}) - I_{\text{c,b}}(\lambda_{\text{em}})) \lambda_{\text{em}} d\lambda_{\text{em}}$$

For the certification measurements, 6 solid samples were randomly selected and dissolved in absolute ethanol. The absorbance of the measured dye solutions at the excitation wavelength of 600 nm was 0.105. Each dye solution was measured 15 times.

### Metrological Traceability

The certified fluorescence quantum yield  $\Phi_{\text{f}}$  value of CRM BAM-F021 was determined with a calibrated custom-made integrating sphere setup. The calibration of the wavelength-dependent spectral responsivity of the integrating sphere setup was performed with the certified spectral radiance standard 10W BN-9701 (SDS, serial number 973372-02) calibrated by PTB Berlin in the wavelength region from 280 nm to 1750 nm (Calibration Certificate No. 73114 PTB 17; Oct. 2017), and with the deuterium lamp LSB 215 (D2, serial number H61685) from LOT Quantum Design GmbH, calibrated by Heraeus Noblelight GmbH for the wavelength range from 200 nm to 400 nm (Test report No. 21966\_2). The spectral responsivity  $s(\lambda)$  of the integrating sphere-detector ensemble was obtained by

dividing the measured signals  $\bar{I}^{raw}(\lambda)$  by the certified spectral radiance ( $\text{Wm}^{-3}\text{sr}^{-1}$ ) of the SDS or deuterium lamp  $Z_{RS}(\lambda)$  (see equation [4]), which are traceable to the International System of Units (SI).

$$s(\lambda) = \frac{\bar{I}^{raw}(\lambda)}{Z_{RS}(\lambda)} \quad [4]$$

## References

1. GUM, Evaluation of measurement data – Guide to the expression of uncertainty in measurement. *Joint Committee for Guides in Metrology, Working Group 1* **2008**.
2. Wuerth, C.; Grabolle, M.; Pauli, J.; Spieles, M.; Resch-Genger, U., Relative and absolute determination of fluorescence quantum yields of transparent samples. *Nature Protocols* **2013**, *8* (8), 1535-1550.
3. Wuerth, C.; Lochmann, C.; Spieles, M.; Pauli, J.; Hoffmann, K.; Schuettrigkeit, T.; Franzl, T.; Resch-Genger, U., Evaluation of a Commercial Integrating Sphere Setup for the Determination of Absolute Photoluminescence Quantum Yields of Dilute Dye Solutions. *Appl. Spectrosc.* **2010**, *64* (7), 733-741.
4. Wuerth, C.; Pauli, J.; Lochmann, C.; Spieles, M.; Resch-Genger, U., Integrating Sphere Setup for the Traceable Measurement of Absolute Photoluminescence Quantum Yields in the Near Infrared. *Anal. Chem.* **2012**, *84* (3), 1345-1352.

## Technical Report

A detailed technical report describing the analysis procedures and the treatment of the analytical data used to certify BAM-F021 is available upon request.

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Bundesanstalt für Materialforschung und -prüfung (BAM)

Dr. S. Richter  
Committee for Certification

Dr. J. Pauli/ Dr. U. Resch-Genger  
Project Coordinators

Specimen

This reference material is supplied by:

Bundesanstalt für Materialforschung und -prüfung (BAM)  
Richard-Willstätter-Str. 11, D-12489 Berlin, Germany

Phone : +49 30 8104 2061

E-Mail: [sales.crm@bam.de](mailto:sales.crm@bam.de)

Fax : +49 30 8104 72061

Internet: [www.webshop.bam.de](http://www.webshop.bam.de)