

1. Resch-Genger, U.; DeRose, P.; Bremser, W.; Ebert, B.; Zwinkels, J.; Pfeifer, D.; Voigt, J.; Taubert, R. D.; Monte, C.; MacDonald, R.; Hollandt, J.; Gauthier, F.; Spieles, M.; Hoffmann, A., Anal. Chem. 2012, 84, 3889-3898.
2. Resch-Genger, U.; DeRose, P.; Bremser, W.; Ebert, B.; Zwinkels, J.; Pfeifer, D.; Voigt, J.; Taubert, R. D.; Monte, C.; MacDonald, R.; Hollandt, J.; Gauthier, F.; Spieles, M.; Hoffmann, A., Anal. Chem. 2012, 84, 3899-3907.
3. Pfeifer, D.; Hoffmann, K.; Hoffmann, A.; Monte, C.; Resch-Genger, U., J. Fluoresc. 2006, 16, 581-587.
4. Resch-Genger, U.; Hoffmann, K.; Nietfeld, W.; Engel, A.; Neukammer, J.; Nitschke, R.; Ebert, B.; Macdonald, R., J. Fluoresc. 2005, 15, 337-362
5. Hollandt, J.; Taubert, R. D.; Seidel, J.; Resch-Genger, U.; Gugg-Helminger, A.; Pfeifer, D.; Monte, C., J. Fluoresc. 2005, 15, 301-313.
6. Resch-Genger, U.; Pfeifer, D.; Pilz, W.; Monte, C.; Hoffmann, A.; Spieles, M.; Rurack, K.; Hollandt, J.; Taubert, R. D.; Schönenberger, B.; Nording, P.; J. Fluoresc. 2005, 15, 315-336.
7. Hoffmann, K.; Monte, C.; Pfeifer, D.; Resch-Genger, U.; G.I.T. Laboratory Journal Europe 6, 2005, 29-31.
8. Monte, C.; Resch-Genger, U.; Pfeifer, D.; Taubert, R. D.; Hollandt, J., Metrologia 2006, 43, 89-93.
9. DeRose, P. C.; Resch-Genger, U., Anal. Chem. 2010, 82, 2129-2133.
10. Resch-Genger, U.; DeRose, P. C., Pure Appl. Chem. 2012, 84, 1815-1835.
11. Resch-Genger, U.; DeRose, P. C., Pure Appl. Chem. 2010, 82, 2315-2335.
12. Grabolle, M.; Spieles, M.; Gaponik, N.; Lesnyak, V.; Eychmüller, A.; Resch-Genger, U., Anal. Chem. 2009, 81, 6285-6294.
13. Würth, C.; Grabolle, M.; Pauli, J.; Spieles, M.; Resch-Genger, U., Anal. Chem. 2011, 83, 3431-3439.
14. Würth, C.; Pauli, J.; Lochmann, C.; Spieles, M.; Resch-Genger, U., Anal. Chem. 2012, 84, 1345-1352.
15. Würth, C.; Grabolle, M.; Pauli, J.; Spieles, M.; Resch-Genger, U., Nature Protocols 2013, 8, 1535.

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Sicherheit in Technik und Chemie



**CERTIFIED REFERENCE  
MATERIALS**  
**BAM-F001x, BAM-F002x,  
BAM-F003x, BAM-F004x  
AND BAM-F005x**

Calibration Kit – Spectral Fluorescence Standards

# BAM-F001x - BAM-F005x

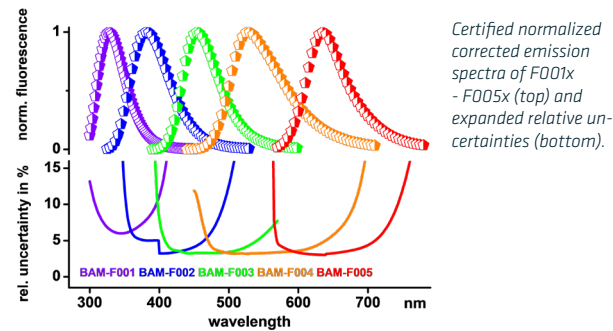
- Five spectral fluorescence standards F001x, F002x, F003x, F004x, and F005x which cover the spectral region of 300 nm to 760 nm as a set.
- Corrected emission spectra of F001x - F005x and corresponding wavelength-dependent expanded relative uncertainties. Certification was performed according to ISO Guide 35 and calculation of the wavelength-dependent uncertainties according to the Guide to the Expression of Uncertainty (GUM).
- Data evaluation software LINKCORR developed from BAM available as download. This includes the certificate files BAMxxxxMx.CTF, the certificates and instructions for use of F001x - F005x with LINKCORR. LINKCORR calculates the relative spectral sensitivity  $s(\lambda)$  of the fluorescence measuring system to be calibrated from the certified emission spectra of the spectral fluorescence standards measured with this instrument and the BAM-certified emission spectra following an evaluated procedure [1,2].

## Use

Addition of a defined amount of absolute ethanol to each ready-made solid dye yields a dye solution that can be measured without additional dilution steps.

## Certified Properties

Normalized corrected emission spectra of F001x - F005x in ethanol obtained with calibrated BAM spectrofluorometer at  $T = 25^\circ\text{C}$ . The emission spectra are provided traceable to the spectral radiance scale and referenced to the spectral photon radiance.

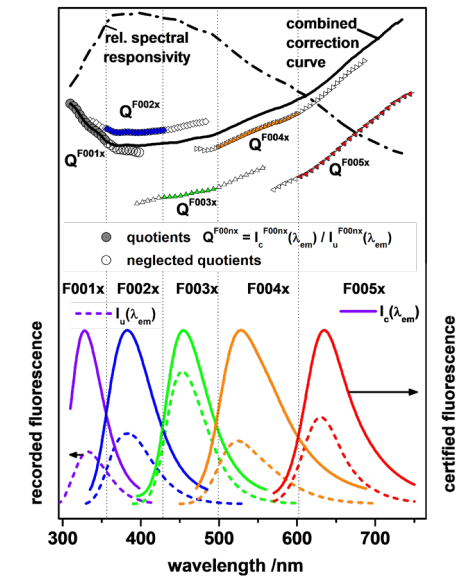


The Calibration Kit was evaluated in an interlaboratory comparison of the National Metrological Institutes NIST (National Institute of Standards and Technology, USA), NRC (National Research Council, Canada), PTB (Physikalisch-Technische Bundesanstalt, Germany), and BAM [1]. Moreover, its performance as calibration tool was assessed in a study with 15 field laboratories arbitrarily chosen from industry and academia [2].

## Area of Application

Calibration of fluorescence instruments and determination of the relative spectral responsivity  $s(\lambda)$  of fluorescence measuring systems from 300 nm to 770 nm under routine measurement conditions [3]. Knowledge of this quantity is mandatory for the correction of emission spectra for distorting instrument-specific effects, thereby yielding instrument-independent “comparable” spectra [1-14] and for the determination of fluorescence quantum yields [12-15]. Regular determination of  $s(\lambda)$  provides a tool for evaluating the longterm stability of the emission channel of fluorescence instruments [3,7,9-11].

Determination of the relative spectral responsivity  $s(\lambda)$  of a fluorescence instrument with BAM-F001x - BAM-F005x and LINKCORR



Bottom: Certified normalized corrected emission spectra (solid lines;  $I_c(I_{em})$ ) of the kit components and uncorrected, i.e., instrument-dependent emission spectra (dashed lines;  $I_u(I_{em})$ ), measured with the instrument to be calibrated.

Middle: Individual quotients  $Q^{F00nx} = I_c^{F00nx}(I_{em}) / I_u^{F00nx}(I_{em})$  ( $n=[1..5]$ ) for each kit dye equaling  $1/s(I)$  of the instrument to be calibrated within the spectral region covered by the standard's emission spectrum.

Top: Combined emission correction curve  $1/s(I)$  (solid black line) calculated from the statistically weighted  $Q^{F00nx}$  as well as its reciprocal  $s(I)$  (black dash-dotted line).