SIGMA-ALDRICH Fluka BAM

Federal Institute for Materials Research and Testing

Standard Operation Procedure (SOP) for Use of

CERTIFIED REFERENCE MATERIALS

BAM-F001, BAM-F002, BAM-F003, BAM-F004 and BAM-F005

Calibration Kit *SPECTRAL FLUORESCENCE STANDARDS*

for the Determination of the Relative Spectral Responsivity s(λ) of Fluorescence Instruments

KIT CONTENT

- 5 dyes **BAM-F001 - BAM-F005**, covering the spectral region of 300 nm to 770 nm provided in portions of 5-15 µg in vials.
  BAM-F001 equals Fluka product number #72594, BAM-F002 #23923, BAM-F003 #96158, BAM-F004 #74245, and BAM-F005 #94053.
- 100 ml of **ethanol**.
- **BAM certificate** of the normalized corrected fluorescence emission spectra of the kit dyes in ethanol measured at 25°C, see also CD.
- BAM software ***LINKCORR*** for the generation of a global emission correction curve 1/**1/s(λ)** from measured uncorrected and BAM-certified corrected spectra of BAM-F001 - BAM-F005, see CD.
- Certified corrected emission spectra of BAM-F001, BAM-F002, BAM-F003, BAM-F004, and BAM-F005 as certificate files **BAM507Mx.ctf** measured at a spectral bandpass (x) of the emission monochromator (M) of 1 nm (**BAM507M1.ctf**), 4 nm (**BAM507M4.ctf**), and 8 nm (**BAM507M8.ctf**), see CD. These files are readable by *LINKCORR*.
- **SOP** for the **determination of the emission correction curve (1/s(λ)) and the relative spectral responsivity s(λ) of fluorescence instruments** with BAM-F001 to BAM-F005 and *LINKCORR* including description of *LINKCORR*.

SCOPE AND LIMITATIONS

- Determination of the relative spectral responsivity s(λ) of fluorescence instruments. s(λ) is the reciprocal of the actual output of *LINKCORR*, the inverse relative spectral responsivity 1/ s(λ) termed emission correction curve.
- Determination of corrected instrument-independent fluorescence emission spectra that are corrected for s(λ) and are accordingly comparable across instruments.
- Characterization of the long-term stability of the emission channel fluorescence instruments by regular determination of 1/s(λ) or s(λ). Fluorescence spectra that are corrected accordingly do not contain contributions from aging of optical components in the emission channel and are thus time-independent.
  For more information, see *certificate* and *certification report*.

STORAGE CONDITIONS, TESTED PROPERTIES, and MEASUREMENT CONDITIONS

See *certificate* and *certification report*.

INSTRUCTIONS FOR USE

See *Scheme1/Schema 1*, SOP *USE OF BAM-F001 - BAM-F005*, SOP *USE OF LINKCORR*, and *certificate*.

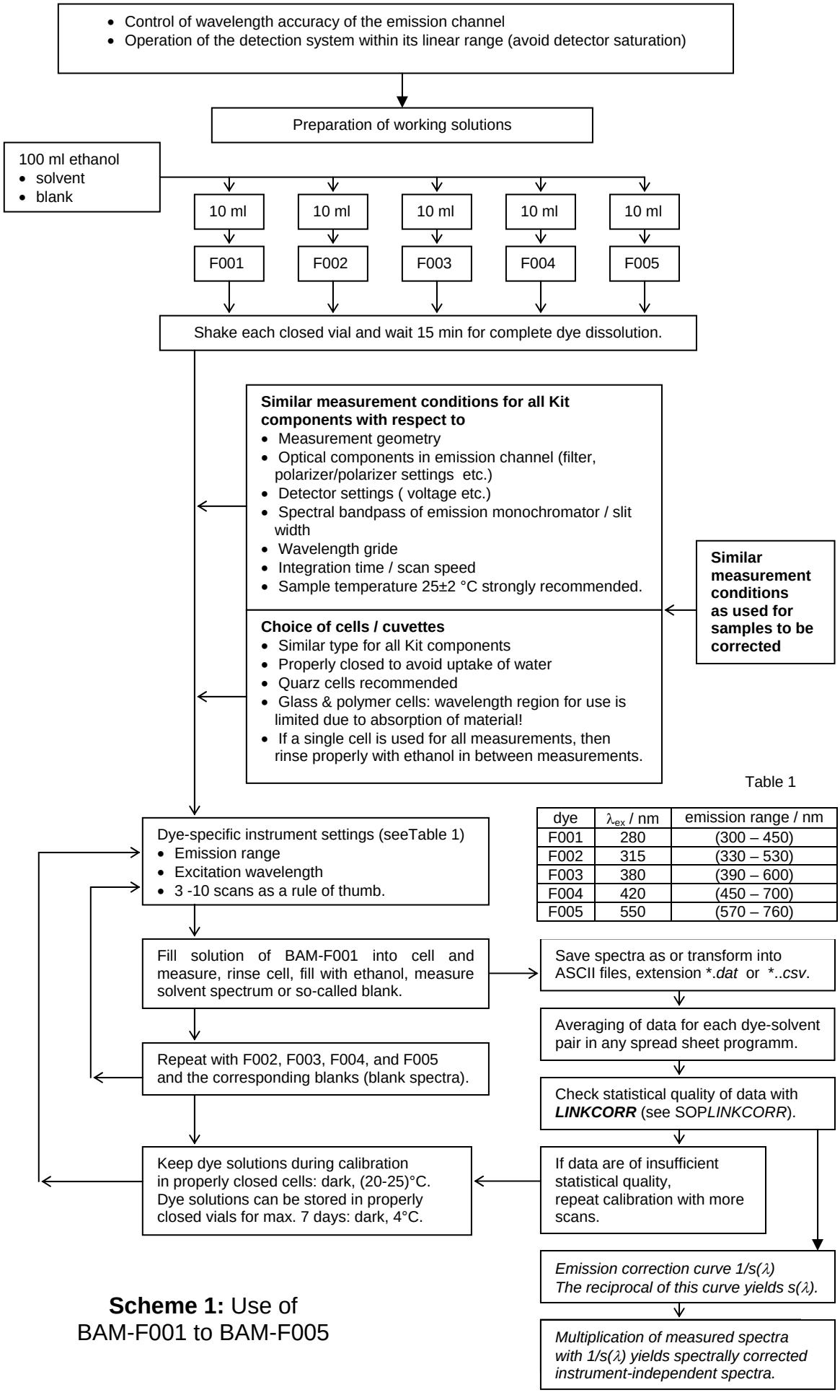
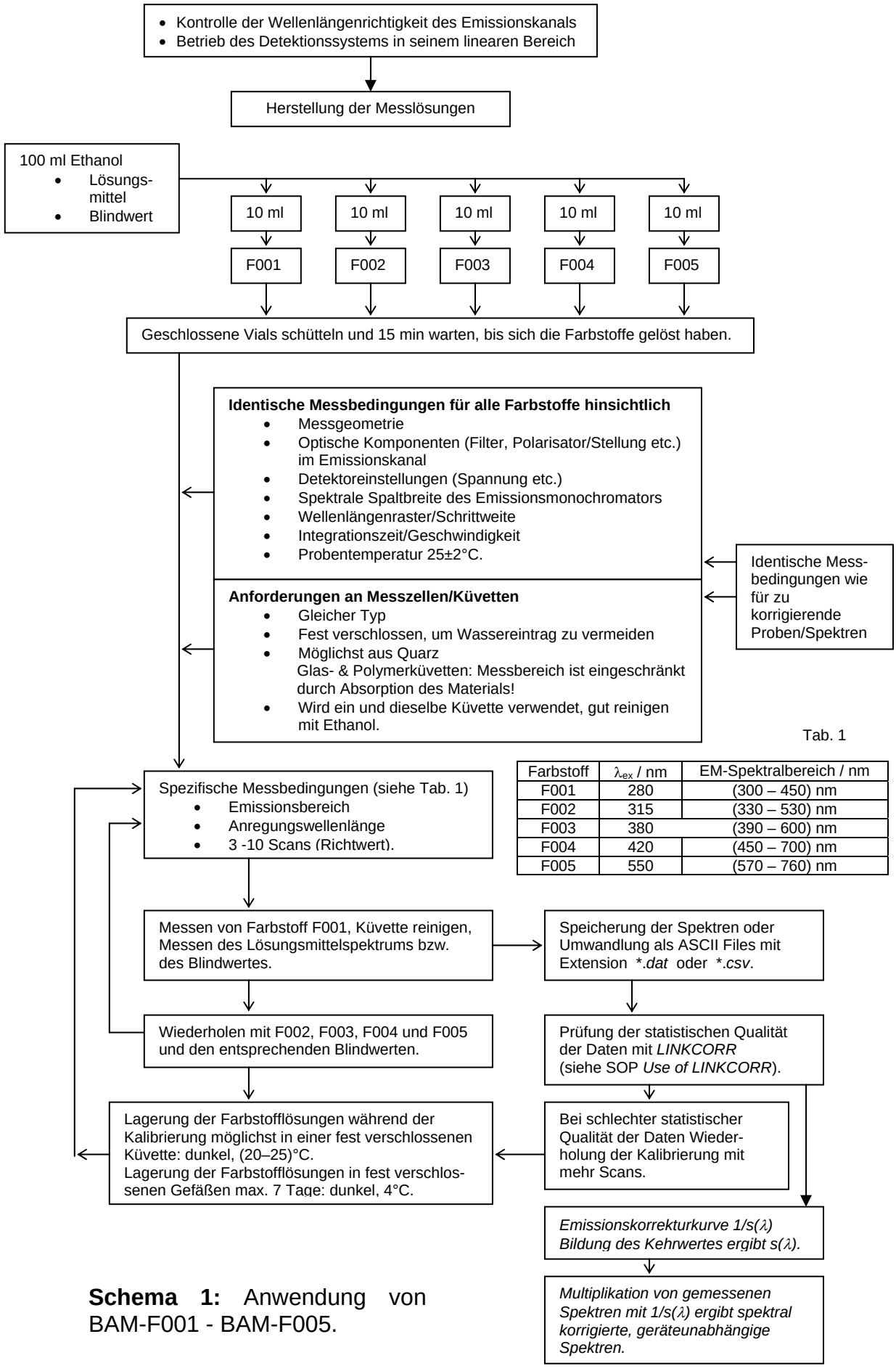


Table 1

dye	λ_{ex} / nm	emission range / nm
F001	280	(300 – 450)
F002	315	(330 – 530)
F003	380	(390 – 600)
F004	420	(450 – 700)
F005	550	(570 – 760)

Scheme 1: Use of BAM-F001 to BAM-F005



Tab. 1

Farbstoff	λ_{ex} / nm	EM-Spektralbereich / nm
F001	280	(300 – 450) nm
F002	315	(330 – 530) nm
F003	380	(390 – 600) nm
F004	420	(450 – 700) nm
F005	550	(570 – 760) nm

Schema 1: Anwendung von BAM-F001 - BAM-F005.

Determination of the Emission Correction Curve (1/s(λ)) and the Relative Spectral Responsivity s(λ) of Fluorescence Instruments with BAM-F001 - BAM-F005 and *LINKCORR*

1. ***Prerequisites.*** Prerequisites for the use of BAM-F001 to BAM-F005 are control and consideration of the wavelength accuracy of the emission channel and operation of the instrument's detection system within its linear range to avoid spectral distortions due to detector saturation, see also *certificate*.
   Measurement of the emission spectra of BAM-F001 - BAM-F005 is recommended at (25±2)°C with thermally equilibrated and properly closed samples using the same type of cuvettes, preferentially quartz cells, for all kit components and the measurement conditions given in *Scheme 1*/*Schema 1* and in the *certificate*. Uptake of water and evaporation of solvent are to be minimized.

2. ***Measurement of emission spectra of the Kit solutions*.** The emission spectra of the Kit solutions filled into clean and properly closed fluorescence cuvettes should be recorded under identical conditions as the samples to be spectrally corrected, see *Scheme 1/Schema 1* and *certificate*. This includes e.g. choice of optical components in the emission channel, measurement geometry as well as settings of the spectral bandpass of the emission monochromator and detector. The excitation wavelength, spectral bandpass of the excitation monochromator, and scanned spectral emission range typically differ in between standards and samples.
   All the fluorescence standards need to be measured under identical conditions including identical wavelength grids (step widths) for the calculation of the global emission correction curve with *LINKCORR*. The conditions, for which the fluorescence standards are optimized, are given in Table 1 in *Scheme 1/Schema 1* as well as in the *certificate* (see Table 6).
   To check on the suitability of the chosen instrument settings, recording of test spectra is recommended. Avoid saturation of the detection system. For most conventional detectors, the strongest fluorescence signal is to be expected from BAM-F003.
   Check *section 5* to make sure that data are recorded in a format readable by *LINKCORR*.
   If not all the components of the Calibration Kit are used, the chosen dyes have to show a spectral overlap such as e.g. BAM-F003, BAM-F004, and BAM-F005. Combinations like e.g. BAM-F001, BAM-F003, and BAM-F005 are rejected by *LINKCORR*.

3. ***Number of scans/averaging of data***. An emission correction curve of sufficient statistical quality can require several emission scans and subsequent averaging to reduce noise. As a rule of thumb, the number of scans performed with the Kit dyes should exceed those run for conventional fluorescence measurements (identical instrument settings) by a factor of at least 3.

4. ***Blank correction (BC)***. A blank correction is performed to remove scattering and fluorescence from the solvent and dark counts of the detector from the spectra of the Kit dyes by subtraction of the solvent or blank spectra from the measured dye spectra. For the recommended blank correction, the emission spectra of the solvent ethanol need to be measured under identical conditions as used for the Kit dyes. The recommended measuring cycle is *BAM-F001*, followed by *blank spectrum BAM-F001* (i.e. solvent measured under conditions as used for dye F001), *dye BAM-F002*, *blank spectrum BAM-F002* etc., running several scans for each dye-solvent pair if necessary.

5. ***Evaluation of data***. The measured (averaged) emission spectra of the Kit components and respective blank or solvent spectra need to be saved in *ASCII file format* for calculation of the global emission correction curve 1/s(λ) with *LINKCORR*. The working principle of *LINKCORR* is described in the *certificate* and in the SOP *Use of BAM-F001 - BAM-F005 for the Determination of the Relative Spectral Responsivity of s(λ) of Fluorescence Instruments*, section SOP *Use of LINKCORR* provided on CD.

Do not normalize or smooth measured data.
Make sure, that the ASCII-Files have the extension *.DAT* or *.CSV* and do not contain non ASCII-signs, binary code elements or extra lines (no data) at the beginning, within or at the end of a file. *LINKCORR* interprets the first data row as wavelength and the second as fluorescence intensity.
LINKCORR can handle at maximum two files per Kit component, i.e., one dye and one blank spectrum with identical emission ranges. If several scans are performed, the corresponding spectra (dye and blank, respectively) need to be averaged prior to the use of *LINKCORR.* If the blank spectra are subtracted, this has to be performed for all (averaged) dye-solvent pairs. In this case, *LINKCORR* works with only five files, i.e., one file per dye.

6. ***Use of LINKCORR***. Start the Windows interface program ***LINKCORRGUI to LINKCORR*** provided on CD by *DOUBLE CLICKING* the icon in the CD root directory. *MARK* the certificate file **BAM507Mx.CTF** where x closely matches the spectral bandpass of the emission monochromator used for your measurement to minimize calibration uncertainties. *SELECT* the measured ASCII-files with *OPEN SPEC* from any directory of your computer. With CALCULATE, the global emission correction curve 1/s(λ) is automatically determined (as**.TXT file*) from the measured emission spectra (including blank spectra) and the certified corrected emission spectra of the Kit dyes, see Figure 1. The resulting emission correction curve is also statistically evaluated. If the results for one or more dyes are of insufficient statistical quality, the respective measurement(s) should be repeated with an increased number of scans as recommended by *LINKCORR*. *LINKCORR* creates a file *CORRF*.TXT* representing the ***emission correction curve 1/s(λ)***. This file consists of two columns with the first column containing the wavelength values and the second one the calculated correction factors.
A more detailed description follows from the SOP *Use of LINKCORR*.

7. ***Application of the emission correction curve.*** *Corrected emission spectra* are obtained by *multipliying* the measured blank corrected spectra with the overall emission correction curve 1/s(λ) (values in column "*CORRFACTOR*" in the file *CORRF*.TXT* created by *LINKCORR*) using a spread-sheet program such as e.g. *MICROCAL ORIGIN* or *MS EXCEL*. Make sure only values that correspond to similar wavelengths are multiplied.

8. **Determination of the relative spectral responsivity s(λ)**. The reciprocal of the emission correction curve yields s(λ).