National Institute of Standards & Technology

Certificate

Standard Reference Material® 2942

Relative Intensity Correction Standard for Fluorescence Spectroscopy: Ultraviolet Emission

This Standard Reference Material (SRM) is intended for use in the evaluation and calibration of the relative spectral responsivity of steady-state fluorescence spectrometers with a continuous excitation source and for determining the day-to-day or instrument-to-instrument intensity variations of a single or similar fluorescence instrument(s), respectively. A unit of SRM 2942 consists of a single cuvette-shaped piece of solid glass.

Certified Values: This SRM is certified for the relative, corrected emission spectrum, E, in relative energy units from emission wavelengths λ_{EM} = 320 nm to 430 nm at 1 nm wavelength intervals at a fixed excitation wavelength (λ_{EX}) of 310.1 nm. Due to larger signal-to-noise levels near the peak maximum, the emission range from λ_{EM} = 320 nm to 390 nm is recommended as optimal for most instruments and applications. The certified values for E and corresponding total uncertainties at the 95 % confidence level, U_{95}, at each emission wavelength are given in Table 1. The values in Table 1 were certified at 25.0 °C ± 0.5 °C with an excitation bandwidth ($\Delta\lambda_{EX}$) of 3.0 nm and an emission bandwidth ($\Delta\lambda_{EM}$) of 3.0 nm. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1]. Metrological traceability of E is to the NIST spectral radiance scale, as expressed in relative energy units. Metrological traceability of wavelength is to the SI unit of meters

Reference Values: This SRM's certified values become reference values when used for spectral correction of fluorescence spectrometers with pulsed light sources, when the frosted face is used with a front-face or epifluorescence geometry or when the polished faces are used with geometries other than that described in the "Instructions for Use" section. A NIST reference value is a non-certified value that is the best estimate of the true value; however, the value does not meet NIST criteria for certification and is provided with an associated uncertainty that may not include all sources of uncertainty [1].

Information Values: A NIST information value is considered to be a value that will be of interest and use to the SRM user, but insufficient information is available to assess adequately the uncertainty associated with the value or only a limited number of analyses were performed [1]. Information values are provided below for the temperature coefficient of the E value at 330 nm and the fluorescence anisotropy (r) at 330 nm of SRM 2942.

Temperature Coefficient (E at 330 nm) = –0.02 % $°C^{-1}$ (range: 11 °C to 39 °C)
r (330 nm) = 0.00

Expiration of Certification: The certification of **SRM 2942** is valid, within the measurement uncertainty specified, until **01 June 2025**, provided the SRM is handled in accordance with instructions given in this certificate (see "Instructions for Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Overall direction and coordination of the technical measurements required for certification of this SRM were performed by P.C. DeRose of the NIST Biosystems and Biomaterials Division.

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Certificate Issue Date: 16 November 2023
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Production and certification of this SRM were performed by P.C. DeRose of the NIST Biosystems and Biomaterials Division, M.V. Smith of the Chemical Sciences Division and G.W. Kramer, J.R. Anderson and K.D. Mielenz formerly of NIST.

Statistical consultation was provided by H.-k. Liu and J. Lu of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

NOTICE AND WARNING TO USERS

Handling and Storage: This SRM should be handled only while wearing clean, powder-free plastic (nitrile recommended) or cloth disposable gloves. The SRM should be grasped in an area away from where the excitation beam will be incident upon or the fluorescence will be collected from the SRM. The supplied case should always be used to store the SRM after it has been wrapped in a clean piece of lens paper. The SRM should be stored in a desiccator or other low-humidity environment around room temperature (15.0 °C to 35.0 °C). It should not be exposed to direct sunlight and should be kept in the dark whenever possible. The faces of the SRM can be washed with absolute ethanol and gently dried with lens paper, if necessary. This SRM should not be exposed to light with wavelengths shorter than 280 nm as this will cause significant photodegradation.

INSTRUCTIONS FOR USE[1]

The SRM should be positioned with the excitation beam normal to and centered on one polished face and with the emission being collected from an adjacent polished face at 90 degrees with respect to the excitation beam. The long frosted side should face away from the detection system. Each SRM has its own serial number etched into the top face, which should face up when in use. The frosted face may be used with a front-face or epifluorescence geometry, or the polished faces may be used with geometries different from that prescribed above, however the certified values become reference values in these cases.

For Correction of Detection System Responsivity: Put the SRM at the sample position of the steady-state fluorescence spectrometer using a standard cuvette holder, with the long frosted side facing away from the detection system. The excitation beam should be horizontally centered on the entrance and exit faces of the SRM. Measurements should be taken with the SRM at a temperature of 25.0 °C ± 0.5 °C. Set the excitation and emission bandwidths as close to 3 nm as possible and set the excitation wavelength to 310.1 nm. Scan the emission monochromator from 320 nm to 430 nm using a 1 nm increment. Collect the detection system signal and, if possible, the simultaneous excitation intensity at each point. Correct the measured fluorescence signal for the excitation intensity, if possible, by dividing the former by the latter. Normalize this spectrum by dividing the intensity values at all wavelengths by the intensity value at 330 nm. Divide each certified value by its corresponding normalized, measured value (preferably excitation intensity corrected) to obtain a correction factor for the detection system responsivity at each emission wavelength. For user convenience, a list of the certified values and uncertainties in ASCII format and a Microsoft EXCEL-based program to produce a similar list with a user-specified λ_{EM} range and step size can be downloaded from the data file link at https://shop.nist.gov/ccrz__ProductDetails?sku=2942&cclcl=en_US.

For Use As a Day-to-Day Intensity Standard: Excite the SRM at a wavelength between 280 nm and 320 nm and measure the fluorescence intensity, preferably at the peak maximum, and the excitation intensity, if possible. Day-to-day intensity variations can be determined by periodically measuring the fluorescence intensity (preferably excitation intensity corrected) under the same experimental conditions and comparing the intensity values over time.

Photostability: After irradiating the SRM for more than 25 hours with an ultraviolet source having a nominal intensity of 0.25 mW $cm^{-2}nm^{-1}$ from 300 nm to 380 nm, no change in the absolute intensity or shape of the emission spectrum was observed within an uncertainty of ± 0.5 % (k = 2) at the peak maximum. This amount of irradiation corresponds to about 50 hours of irradiation with our fluorometer's excitation beam under the conditions used for certification.

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[1] Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Physical Description: SRM 2942 is a cerium-doped (a mole fraction of 0.00025 % CeO_2) phosphate matrix glass. Each unit of this SRM is a rectangular solid block with standard cuvette dimensions 12.5 mm × 12.5 mm × 45.0 mm, with three of the four long faces optically polished and one long face, as well as the top face and bottom faces, ground to a frosted finish using a 400 grit polish. The serial number of each unit is etched on the top face. There are 50 units of SRM 2942 with serial numbers Ce014-63.

Certification Measurements: The excitation and emission monochromators were calibrated for wavelength using one of the Xe source lamp lines and one of the Hg lines of a pen lamp, respectively. A calibrated light source was used to determine the relative responsivity of the detection system as a function of wavelength from 344 nm to 430 nm with the aid of a calibrated reflector at the sample position to reflect the light from the calibrated source into the detection system [2]. For wavelengths from 320 nm to 343 nm, the relative responsivity of the detection system was determined using a two-step method. In the first step, a calibrated detector was used to measure the spectral flux of the excitation beam at the sample from 320 nm to 343 nm. A calibrated diffuse reflector (CR) was then used to reflect the excitation beam into the detection system where the signal was measured, while excitation and emission monochromators were synchronously scanned from 320 nm to 343 nm [2].

The spectrum of each SRM was then collected from an excitation wavelength of 320 nm to 430 nm at 1 nm increments and a fixed excitation wavelength of 310.1 nm. The excitation and emission bandwidths were set to 3 nm and the relative excitation intensity was collected simultaneously with the fluorescence intensity, enabling the measured SRM spectrum to be corrected for variations in excitation intensity. The resulting SRM spectrum was then corrected for the responsivity of the detection system. The certified spectrum is an average of the corrected spectra for all SRM units in this batch, which has also been normalized to one at 330 nm. The absolute peak intensity was also found to vary by 4% or less for all units in this batch.

Assignment of Uncertainties: Standard uncertainty components equivalent to the estimated standard deviation were assigned for sample inhomogeniety, sample variation within the batch, and measurement uncertainties. These values were then combined with systematic uncertainties due to wavelength accuracy, bandwidth accuracy, temperature accuracy, spatial uncertainty of the excitation beam's position on the sample (causing secondary inner filter effect uncertainties), variation of F and G polarization ratios among instruments, and uncertainty in the spectral shape correction (due to uncertainty in the radiance, reflectance, and responsivity values of the calibrated light source, reflector, and detector), using the root-sum-of-squares method. An expansion factor of $k = 2$ was applied so that the expanded uncertainties given in this certificate express an interval within which the true value is expected to fall with a level of confidence of approximately 95 % for a normal distribution [3].

Table 1. Relative Corrected Emission Spectrum of SRM 2942 at λ_{EX} = 310.1 nm

λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}
320	0.7834	0.0574	379	0.1770	0.0138
321	0.8289	0.0596	380	0.1655	0.0129
322	0.8662	0.0617	381	0.1561	0.0121
323	0.8982	0.0639	382	0.1464	0.0114
324	0.9279	0.0663	383	0.1373	0.0107
325	0.9493	0.0690	384	0.1277	0.0099
326	0.9670	0.0722	385	0.1198	0.0093
327	0.9805	0.0759	386	0.1112	0.0087
328	0.9919	0.0799	387	0.1037	0.0081
329	0.9974	0.0840	388	0.0964	0.0076
330	1.0000	0.0877	389	0.0889	0.0071
331	0.9995	0.0906	390	0.0826	0.0066
332	0.9984	0.0925	391	0.0766	0.0062
333	0.9934	0.0930	392	0.0717	0.0058
334	0.9876	0.0924	393	0.0664	0.0054
335	0.9816	0.0907	394	0.0613	0.0050
336	0.9741	0.0882	395	0.0571	0.0047
337	0.9658	0.0852	396	0.0526	0.0043
338	0.9567	0.0822	397	0.0489	0.0040
339	0.9478	0.0795	398	0.0454	0.0037
340	0.9376	0.0773	399	0.0416	0.0035
341	0.9267	0.0756	400	0.0387	0.0032
342	0.9159	0.0747	401	0.0358	0.0030
343	0.9034	0.0746	402	0.0333	0.0028
344	0.8901	0.0751	403	0.0310	0.0026
345	0.8764	0.0760	404	0.0286	0.0024
346	0.8619	0.0764	405	0.0266	0.0022
347	0.8459	0.0760	406	0.0247	0.0021
348	0.8290	0.0744	407	0.0230	0.0019
349	0.8107	0.0720	408	0.0212	0.0018
350	0.7908	0.0689	409	0.0198	0.0017
351	0.7696	0.0658	410	0.0183	0.0016
352	0.7469	0.0625	411	0.0171	0.0015
353	0.7232	0.0593	412	0.0160	0.0014
354	0.6984	0.0562	413	0.0149	0.0013
355	0.6720	0.0535	414	0.0139	0.0012
356	0.6457	0.0512	415	0.0129	0.0011
357	0.6191	0.0490	416	0.0121	0.0011
358	0.5915	0.0468	417	0.0112	0.0010
359	0.5646	0.0447	418	0.0105	0.0010
360	0.5381	0.0426	419	0.0098	0.0009
361	0.5110	0.0405	420	0.0093	0.0009
362	0.4853	0.0385	421	0.0087	0.0008
363	0.4606	0.0364	422	0.0082	0.0008
364	0.4355	0.0343	423	0.0077	0.0008
365	0.4125	0.0324	424	0.0073	0.0008
366	0.3904	0.0305	425	0.0069	0.0007
367	0.3680	0.0287	426	0.0065	0.0007
368	0.3477	0.0271	427	0.0061	0.0007
369	0.3284	0.0256	428	0.0058	0.0007
370	0.3090	0.0243	429	0.0055	0.0006
371	0.2914	0.0230	430	0.0052	0.0006
372	0.2754	0.0217			
373	0.2604	0.0205			
374	0.2459	0.0194			
375	0.2311	0.0182			
376	0.2157	0.0170			
377	0.2027	0.0159			
378	0.1897	0.0148			

REFERENCES

[1] Beauchamp, C.R.; Camara, J.E.; Carney, J.; Choquette, S.J.; Cole, K.D.; DeRose, P.C.; Duewer, D.L.; Epstein, M.S.; Kline, M.C.; Lippa, K.A.; Lucon, E.; Molloy, J.; Nelson, M.A.; Phinney, K.W.; Polakoski, M.; Possolo, A.; Sander, L.C.; Schiel, J.E.; Sharpless, K.E.; Toman, B.; Winchester, M.R.; Windover, D.; *Metrological Tools for the Reference Materials and Reference Instruments of the NIST Material Measurement Laboratory*; NIST Special Publication 260-136, 2021 edition; U.S. Government Printing Office: Washington, DC (2021); available at https://nvlpubs.nist.gov/nistpubs/SpecialPublications/NIST.SP.260-136-2021.pdf (accessed Nov 2023).

[2] DeRose, P.C.; Early, E.A.; Kramer, G.W.; *Qualification of a Fluorescence Spectrometer for Measuring True Fluorescence Spectra*; In Proceedings of The 5th Oxford Conference on Spectrophotometry, Crown, UK (2008).

[3] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (JCGM) (2008); available at https://www.bipm.org/en/committees/jc/jcgm/publications (accessed Nov 2023); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at https://www.nist.gov/pml/nist-technical-note-1297 (accessed Nov 2023).

Certificate Revision History: 16 November 2023 (Change of expiration date; editorial changes); 01 February 2019 (Change of expiration date; editorial changes); 11 June 2009 (Original certificate date).