National Institute of Standards & Technology

Certificate

Standard Reference Material® 2941

Relative Intensity Correction Standard for Fluorescence Spectroscopy: Green Emission

Series Sample

This Standard Reference Material (SRM) is intended for use for 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. This SRM is certified for the relative, corrected emission spectrum, E, in relative energy units from emission wavelengths λ_{EM} = 450 nm to 650 nm at 1 nm wavelength intervals at a fixed excitation wavelength (λ_{EX}) of 427 nm. **Note:** These standard's certified values become reference values when used for spectral correction of fluorescence spectrometers with pulsed light sources. The SRM should be positioned with the excitation beam normal to and centered on one polished face and with the emission being collected from the center of an adjacent polished face at 90° 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. This SRM consists of a single cuvette-shaped piece of solid glass.

Certified Values: NIST certified values are values for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or accounted for by NIST [1,2]. The certified values for this material are listed in Table 1. The values 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. 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. 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: NIST Reference values are non-certified values that are the best estimates of the true values; however, the values do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision and may not include all sources of uncertainty.

Expiration of Certification: The certification of **SRM 2941** is valid, within the measurement uncertainty specified, until **01 January 2023**, provided the SRM is handled and stored in accordance with the instructions given here (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 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 G.W. Kramer formerly of NIST.

Production and certification of this SRM were performed by P.C. DeRose, M.V. Smith, and D.H. Blackburn of the NIST Biosystems and Biomaterials Division. Assistance was provided by E.A. Early and K.D. Mielenz formerly of NIST, and D.L. Duewer of the NIST Chemical Sciences Division.

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.

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Certificate Issue Date: 23 February 2018
Certificate Revision History on Last Page

Table 1. Certified Relative Corrected Emission Spectrum of SRM 2941 Series Sample at λ_{EX} = 427 nm

λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}
450	0.0004	0.0001	509	0.8411	0.0442	568	0.3729	0.0184	627	0.0417	0.0027
451	0.0005	0.0001	510	0.8433	0.0433	569	0.3634	0.0182	628	0.0400	0.0027
452	0.0005	0.0001	511	0.8444	0.0427	570	0.3531	0.0177	629	0.0386	0.0025
453	0.0006	0.0001	512	0.8454	0.0419	571	0.3435	0.0174	630	0.0373	0.0025
454	0.0007	0.0001	513	0.8416	0.0403	572	0.3354	0.0174	631	0.0359	0.0025
455	0.0009	0.0002	514	0.8433	0.0405	573	0.3269	0.0170	632	0.0346	0.0023
456	0.0010	0.0002	515	0.8474	0.0403	574	0.3188	0.0170	633	0.0335	0.0023
457	0.0012	0.0002	516	0.8550	0.0390	575	0.3112	0.0171	634	0.0322	0.0022
458	0.0014	0.0003	517	0.8632	0.0393	576	0.3038	0.0167	635	0.0311	0.0022
459	0.0017	0.0003	518	0.8777	0.0412	577	0.2953	0.0161	636	0.0300	0.0021
460	0.0020	0.0004	519	0.8930	0.0404	578	0.2872	0.0163	637	0.0291	0.0020
461	0.0024	0.0005	520	0.9098	0.0404	579	0.2784	0.0153	638	0.0281	0.0019
462	0.0029	0.0006	521	0.9316	0.0409	580	0.2694	0.0152	639	0.0272	0.0019
463	0.0035	0.0007	522	0.9501	0.0424	581	0.2599	0.0147	640	0.0262	0.0019
464	0.0043	0.0009	523	0.9686	0.0433	582	0.2503	0.0145	641	0.0254	0.0017
465	0.0052	0.0011	524	0.9826	0.0424	583	0.2406	0.0137	642	0.0245	0.0016
466	0.0062	0.0013	525	0.9941	0.0433	584	0.2312	0.0133	643	0.0236	0.0017
467	0.0075	0.0016	526	1.0000	0.0423	585	0.2220	0.0127	644	0.0228	0.0015
468	0.0090	0.0018	527	1.0006	0.0416	586	0.2125	0.0122	645	0.0220	0.0015
469	0.0109	0.0022	528	0.9972	0.0422	587	0.2031	0.0115	646	0.0213	0.0015
470	0.0129	0.0026	529	0.9878	0.0418	588	0.1943	0.0108	647	0.0205	0.0014
471	0.0153	0.0030	530	0.9726	0.0412	589	0.1857	0.0104	648	0.0198	0.0014
472	0.0184	0.0036	531	0.9564	0.0415	590	0.1779	0.0097	649	0.0189	0.0013
473	0.0216	0.0041	532	0.9347	0.0403	591	0.1697	0.0095	650	0.0182	0.0012
474	0.0257	0.0048	533	0.9119	0.0390	592	0.1625	0.0091			
475	0.0303	0.0055	534	0.8866	0.0380	593	0.1559	0.0086			
476	0.0353	0.0062	535	0.8604	0.0371	594	0.1498	0.0082			
477	0.0416	0.0070	536	0.8387	0.0361	595	0.1439	0.0076			
478	0.0486	0.0080	537	0.8160	0.0352	596	0.1386	0.0074			
479	0.0566	0.0089	538	0.7955	0.0353	597	0.1336	0.0071			
480	0.0660	0.0100	539	0.7769	0.0344	598	0.1289	0.0067			
481	0.0762	0.0111	540	0.7598	0.0340	599	0.1246	0.0064			
482	0.0885	0.0122	541	0.7475	0.0336	600	0.1205	0.0064			
483	0.1026	0.0135	542	0.7348	0.0325	601	0.1168	0.0062			
484	0.1172	0.0148	543	0.7250	0.0323	602	0.1133	0.0061			
485	0.1338	0.0161	544	0.7171	0.0319	603	0.1095	0.0056			
486	0.1511	0.0174	545	0.7109	0.0316	604	0.1063	0.0054			
487	0.1704	0.0186	546	0.7048	0.0312	605	0.1027	0.0053			
488	0.1916	0.0199	547	0.6993	0.0309	606	0.0997	0.0052			
489	0.2134	0.0212	548	0.6940	0.0310	607	0.0966	0.0051			
490	0.2385	0.0225	549	0.6859	0.0300	608	0.0932	0.0048			
491	0.2661	0.0241	550	0.6775	0.0300	609	0.0902	0.0047			
492	0.2940	0.0253	551	0.6672	0.0303	610	0.0867	0.0045			
493	0.3262	0.0270	552	0.6565	0.0288	611	0.0835	0.0044			
494	0.3608	0.0289	553	0.6426	0.0290	612	0.0804	0.0045			
495	0.3976	0.0308	554	0.6264	0.0280	613	0.0773	0.0042			
496	0.4382	0.0329	555	0.6092	0.0278	614	0.0742	0.0041			
497	0.4811	0.0345	556	0.5894	0.0262	615	0.0709	0.0040			
498	0.5232	0.0361	557	0.5688	0.0252	616	0.0679	0.0038			
499	0.5684	0.0380	558	0.5477	0.0244	617	0.0649	0.0038			
500	0.6135	0.0399	559	0.5268	0.0232	618	0.0620	0.0038			
501	0.6546	0.0416	560	0.5077	0.0226	619	0.0591	0.0035			
502	0.6955	0.0432	561	0.4866	0.0219	620	0.0567	0.0034			
503	0.7341	0.0445	562	0.4667	0.0210	621	0.0542	0.0033			
504	0.7641	0.0448	563	0.4484	0.0203	622	0.0517	0.0032			
505	0.7919	0.0455	564	0.4304	0.0195	623	0.0496	0.0031			
506	0.8131	0.0450	565	0.4145	0.0195	624	0.0475	0.0031			
507	0.8270	0.0455	566	0.3996	0.0187	625	0.0454	0.0030			
508	0.8351	0.0441	567	0.3857	0.0183	626	0.0435	0.0029			

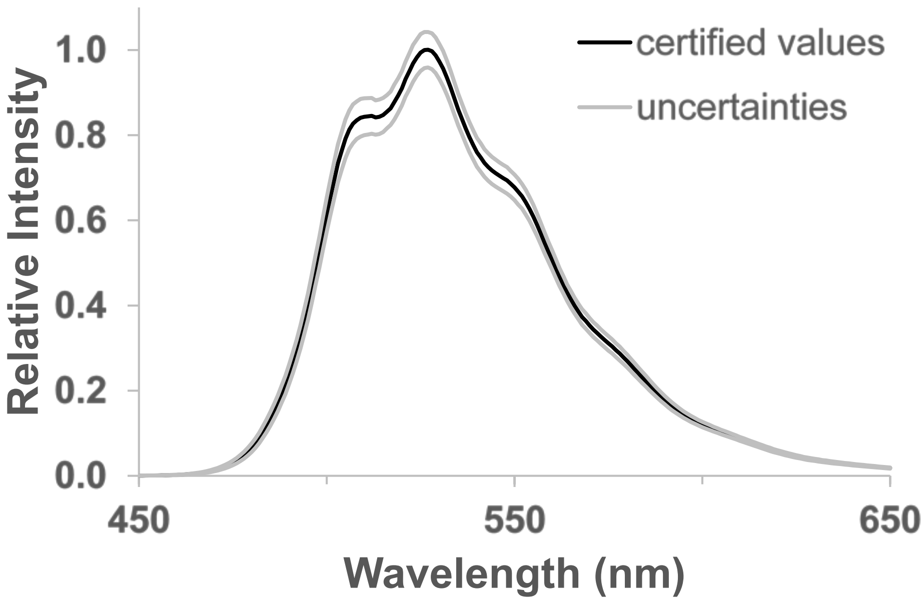

Figure 1. Certified Spectrum for SRM 2941 Sample Series.

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 [1,2]. A NIST information value is provided for information purposes only. Information values for the relative temperature coefficient of the E value at 526 nm and the fluorescence anisotropy (r) at 526 nm of SRM 2941 are listed in Table 2. Information values cannot be used to establish metrological traceability.

Table 2: Temperature Coefficient of the E Value at 526 nm and the Fluorescence Anisotropy (r) at 526 nm of SRM 2941

Relative Temperature Coefficient for E (at 526 nm):	–1.31 % °C^{-1} (range: 11 °C to 39 °C)
Fluorescence Anisotropy (r) at 526 nm:	0.055

Physical Description: SRM 2941 is a depleted uranium-doped (0.01 % U_3O_8 by weight) borate 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, the top face and the bottom face ground to a frosted finish using a 400-grit polish. The serial number of each unit is etched on the top face.

Photostability: After irradiating the SRM with a white light source with a nominal intensity of 13 mW cm^{-2} nm^{-1} from 400 nm to 700 nm for more than 17 hours, no change in the absolute intensity or shape of the emission spectrum was observed within an uncertainty of ± 0.4 % (k = 2) at the peak maximum. This amount of irradiation corresponds to about 242 hours of irradiation with our fluorometer's excitation beam under the conditions used for certification.

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 with the aid of a calibrated reflector at the sample position to reflect the light from the calibrated source into the detection system [3]. The spectrum of each SRM was then collected from an emission wavelength of 450 nm to 650 nm at 1 nm increments and a fixed excitation wavelength of 427 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 and a small emission wavelength bias. The certified spectrum shown in Figure 1 is an average of the corrected spectra for all SRM units in this batch, which has also been normalized to one at 526 nm. The absolute peak intensity was also found to vary by less than 2 % for all units in this batch.

Assignment of Uncertainties: Standard uncertainty components equivalent to the estimated standard deviation were assigned for sample inhomogeneity, 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 [4] among instruments, and uncertainty in the spectral shape correction (due to uncertainty in the radiance and reflectance values of the calibrated light source and reflector), 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 ($E \pm U_{95}$) within which the true value is expected to fall with a level of confidence of approximately 95 % for a normal distribution [2].

Handling and Storage: This SRM should be handled only while wearing a pair of clean, powder-free plastic (nitrile recommended) or cloth disposable gloves. The SRM should be grasped with two fingers in an area away from where the excitation beam will be incident on or where 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.

INSTRUCTIONS FOR USE

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 427 nm. Scan the emission monochromator from 450 nm to 650 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 526 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://www-s.nist.gov/srmors/view_detail.cfm?srm=2941.

For Day-to-Day Intensity Standard: Excite the SRM at a wavelength between 400 nm and 500 nm, preferably at 427 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.

REFERENCES

[1] May, W.; Parris, R.; Beck II, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136; U.S. Government Printing Office: Washington, DC (2000); available at https://www.nist.gov/sites/default/files/documents/srm/SP260-136.PDF (accessed Feb 2018).

[2] 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 http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Feb 2018); 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 Feb 2018).

[3] DeRose, P.C.; Early, E.A.; Kramer, G.W.; *Qualification of a Fluorescence Spectrometer for Measuring True Fluorescence Spectra*; Rev. Sci. Instrum., Vol. 78 (2007).

[4] Mielenz, K.D.; *Measurement of Photoluminescence*; Mielenz, K.D. Ed., Optical Radiation Measurements, Vol. 3, Academic Press: New York, NY pp. 58-76 (1982).

Certificate Revision History: 23 February 2018 (Change of expiration date; increase of uncertainty values; addition of Figure 1; editorial changes); 18 April 2007 (Original certificate date).

Users of this SRM should ensure that the certificate in their possession is current. This can be accomplished by contacting the SRM Program at: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.