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 power 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:** This 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.

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 this SRM is valid until **18 April 2017**, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given here (see "Instructions for Use").

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) will facilitate notification.

The overall direction and coordination of the technical measurements required for certification of this SRM were performed by G.W. Kramer of the NIST Biochemical Science Division.

Production and certification of this SRM were performed by P.C. DeRose, M.V. Smith, and D.H. Blackburn of the NIST Biochemical Science Division. Assistance was provided by E.A. Early and K.D. Mielenz of the NIST Optical Technology Division and D.L. Duewer of the NIST Analytical Chemistry Division.

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

The support aspects involved in the issuance of this SRM were coordinated through the NIST Measurement Services Division.

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Table 1. Relative Corrected Emission Spectrum of SRM 294 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	0.0374	568	0.3729	0.0164	627	0.0417	0.0020
451	0.0005	0.0001	510	0.8433	0.0374	569	0.3634	0.0160	628	0.0400	0.0020
452	0.0005	0.0001	511	0.8444	0.0366	570	0.3531	0.0155	629	0.0386	0.0018
453	0.0006	0.0001	512	0.8454	0.0367	571	0.3435	0.0150	630	0.0373	0.0018
454	0.0007	0.0001	513	0.8416	0.0358	572	0.3354	0.0147	631	0.0359	0.0018
455	0.0009	0.0001	514	0.8433	0.0360	573	0.3269	0.0144	632	0.0346	0.0016
456	0.0010	0.0001	515	0.8474	0.0365	574	0.3188	0.0141	633	0.0335	0.0018
457	0.0012	0.0001	516	0.8550	0.0358	575	0.3112	0.0139	634	0.0322	0.0016
458	0.0014	0.0002	517	0.8632	0.0361	576	0.3038	0.0135	635	0.0311	0.0016
459	0.0017	0.0002	518	0.8777	0.0387	577	0.2953	0.0130	636	0.0300	0.0015
460	0.0020	0.0002	519	0.8930	0.0385	578	0.2872	0.0131	637	0.0291	0.0015
461	0.0024	0.0003	520	0.9098	0.0389	579	0.2784	0.0121	638	0.0281	0.0014
462	0.0029	0.0003	521	0.9316	0.0398	580	0.2694	0.0121	639	0.0272	0.0014
463	0.0035	0.0004	522	0.9501	0.0413	581	0.2599	0.0113	640	0.0262	0.0014
464	0.0043	0.0005	523	0.9686	0.0426	582	0.2503	0.0114	641	0.0254	0.0012
465	0.0052	0.0006	524	0.9826	0.0420	583	0.2406	0.0106	642	0.0245	0.0012
466	0.0062	0.0006	525	0.9941	0.0431	584	0.2312	0.0103	643	0.0236	0.0013
467	0.0075	0.0008	526	1.0000	0.0422	585	0.2220	0.0098	644	0.0228	0.0011
468	0.0090	0.0009	527	1.0006	0.0416	586	0.2125	0.0095	645	0.0220	0.0012
469	0.0109	0.0011	528	0.9972	0.0422	587	0.2031	0.0092	646	0.0213	0.0011
470	0.0129	0.0012	529	0.9878	0.0417	588	0.1943	0.0085	647	0.0205	0.0010
471	0.0153	0.0015	530	0.9726	0.0410	589	0.1857	0.0082	648	0.0198	0.0011
472	0.0184	0.0017	531	0.9564	0.0413	590	0.1779	0.0079	649	0.0189	0.0010
473	0.0216	0.0020	532	0.9347	0.0399	591	0.1697	0.0078	650	0.0182	0.0010
474	0.0257	0.0023	533	0.9119	0.0386	592	0.1625	0.0075			
475	0.0303	0.0027	534	0.8866	0.0373	593	0.1559	0.0072			
476	0.0353	0.0031	535	0.8604	0.0364	594	0.1498	0.0068			
477	0.0416	0.0036	536	0.8387	0.0355	595	0.1439	0.0064			
478	0.0486	0.0042	537	0.8160	0.0346	596	0.1386	0.0063			
479	0.0566	0.0046	538	0.7955	0.0344	597	0.1336	0.0062			
480	0.0660	0.0054	539	0.7769	0.0335	598	0.1289	0.0058			
481	0.0762	0.0061	540	0.7598	0.0331	599	0.1246	0.0056			
482	0.0885	0.0068	541	0.7475	0.0328	600	0.1205	0.0057			
483	0.1026	0.0077	542	0.7348	0.0316	601	0.1168	0.0055			
484	0.1172	0.0088	543	0.7250	0.0314	602	0.1133	0.0054			
485	0.1338	0.0098	544	0.7171	0.0311	603	0.1095	0.0050			
486	0.1511	0.0109	545	0.7109	0.0308	604	0.1063	0.0049			
487	0.1704	0.0118	546	0.7048	0.0304	605	0.1027	0.0048			
488	0.1916	0.0131	547	0.6993	0.0301	606	0.0997	0.0047			
489	0.2134	0.0142	548	0.6940	0.0302	607	0.0966	0.0046			
490	0.2385	0.0156	549	0.6859	0.0292	608	0.0932	0.0043			
491	0.2661	0.0170	550	0.6775	0.0293	609	0.0902	0.0042			
492	0.2940	0.0182	551	0.6672	0.0296	610	0.0867	0.0039			
493	0.3262	0.0199	552	0.6565	0.0280	611	0.0835	0.0038			
494	0.3608	0.0214	553	0.6426	0.0283	612	0.0804	0.0039			
495	0.3976	0.0235	554	0.6264	0.0272	613	0.0773	0.0036			
496	0.4382	0.0253	555	0.6092	0.0272	614	0.0742	0.0034			
497	0.4811	0.0272	556	0.5894	0.0256	615	0.0709	0.0032			
498	0.5232	0.0287	557	0.5688	0.0246	616	0.0679	0.0032			
499	0.5684	0.0304	558	0.5477	0.0235	617	0.0649	0.0030			
500	0.6135	0.0323	559	0.5268	0.0223	618	0.0620	0.0031			
501	0.6546	0.0334	560	0.5077	0.0217	619	0.0591	0.0028			
502	0.6955	0.0352	561	0.4866	0.0208	620	0.0567	0.0027			
503	0.7341	0.0368	562	0.4667	0.0199	621	0.0542	0.0025			
504	0.7641	0.0365	563	0.4484	0.0190	622	0.0517	0.0025			
505	0.7919	0.0377	564	0.4304	0.0182	623	0.0496	0.0023			
506	0.8131	0.0378	565	0.4145	0.0180	624	0.0475	0.0023			
507	0.8270	0.0382	566	0.3996	0.0169	625	0.0454	0.0022			
508	0.8351	0.0373	567	0.3857	0.0166	626	0.0435	0.0022			

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.

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. There are 13 units of SRM 2941 Series Sample with serial numbers U0XX to U0YY.

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 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 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 [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 ± 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 dessicator 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://srmors.nist.gov/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, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions 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).

[2] ISO; G*uide to the Expression of Uncertainty in Measurement*; ISBN 92-67-10188-9, 1st ed.; International Organization for Standardization: Geneva, Switzerland (1993); 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 http://physics.nist.gov/Pubs/.

[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).

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) 975-6776; fax (301) 926-4751; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.