National Institute of Standards & Technology

Certificate

Standard Reference Material® 2941a

Relative Intensity Correction Standard for Fluorescence Spectroscopy: Green Emission

Lot 1

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 [1,2].

Expiration of Certification: The certification of **SRM 2941a** is valid, within the measurement uncertainty specified, until **01 September 2028**, 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 P.C. DeRose of the NIST Biosystems and Biomaterials Division.

Production and certification of this SRM were performed by P.C. DeRose, and J.R. Anderson and A.V. Kirchhoff of the NIST Fabrication Technology Division.

Statistical consultation was provided by 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.

Sheng Lin-Gibson, Acting Chief
Biosystems and Biomaterials Division

Gaithersburg, MD 20899
Certificate Issue Date: 10 October 2018

Steven J. Choquette, Director
Office of Reference Materials

Table 1. Certified Relative Corrected Emission Spectrum of SRM 2941a Lot 1 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	0.8628	0.0401	568	0.3579	0.0190	627	0.0372	0.0029
451	0.0005	0.0001	510	0.8644	0.0393	569	0.3475	0.0188	628	0.0356	0.0028
452	0.0006	0.0001	511	0.8618	0.0383	570	0.3372	0.0186	629	0.0342	0.0027
453	0.0007	0.0001	512	0.8602	0.0374	571	0.3288	0.0185	630	0.0328	0.0026
454	0.0008	0.0002	513	0.8577	0.0365	572	0.3199	0.0183	631	0.0318	0.0025
455	0.0010	0.0002	514	0.8568	0.0357	573	0.3116	0.0181	632	0.0306	0.0024
456	0.0011	0.0002	515	0.8607	0.0352	574	0.3041	0.0180	633	0.0294	0.0023
457	0.0014	0.0003	516	0.8663	0.0347	575	0.2966	0.0178	634	0.0283	0.0022
458	0.0016	0.0003	517	0.8762	0.0344	576	0.2882	0.0176	635	0.0274	0.0022
459	0.0019	0.0004	518	0.8884	0.0343	577	0.2800	0.0173	636	0.0263	0.0021
460	0.0023	0.0005	519	0.9029	0.0343	578	0.2717	0.0170	637	0.0254	0.0020
461	0.0028	0.0006	520	0.9221	0.0346	579	0.2628	0.0166	638	0.0246	0.0019
462	0.0034	0.0007	521	0.9403	0.0349	580	0.2532	0.0161	639	0.0237	0.0019
463	0.0041	0.0008	522	0.9583	0.0352	581	0.2439	0.0156	640	0.0227	0.0018
464	0.0050	0.0010	523	0.9733	0.0356	582	0.2344	0.0151	641	0.0219	0.0017
465	0.0061	0.0012	524	0.9869	0.0360	583	0.2264	0.0147	642	0.0212	0.0017
466	0.0073	0.0015	525	0.9965	0.0363	584	0.2170	0.0141	643	0.0205	0.0016
467	0.0089	0.0018	526	1.0000	0.0365	585	0.2077	0.0135	644	0.0195	0.0015
468	0.0105	0.0021	527	0.9980	0.0365	586	0.1986	0.0129	645	0.0187	0.0015
469	0.0126	0.0025	528	0.9913	0.0365	587	0.1900	0.0123	646	0.0182	0.0014
470	0.0151	0.0030	529	0.9779	0.0363	588	0.1820	0.0117	647	0.0175	0.0014
471	0.0179	0.0035	530	0.9602	0.0360	589	0.1747	0.0112	648	0.0169	0.0014
472	0.0212	0.0040	531	0.9439	0.0357	590	0.1672	0.0107	649	0.0161	0.0013
473	0.0249	0.0046	532	0.9210	0.0352	591	0.1599	0.0101	650	0.0154	0.0013
474	0.0295	0.0053	533	0.8968	0.0347	592	0.1531	0.0096			
475	0.0347	0.0061	534	0.8711	0.0340	593	0.1471	0.0092			
476	0.0405	0.0069	535	0.8475	0.0335	594	0.1413	0.0088			
477	0.0473	0.0077	536	0.8252	0.0329	595	0.1356	0.0083			
478	0.0548	0.0086	537	0.8008	0.0322	596	0.1307	0.0080			
479	0.0639	0.0097	538	0.7804	0.0317	597	0.1259	0.0076			
480	0.0743	0.0108	539	0.7614	0.0311	598	0.1215	0.0073			
481	0.0857	0.0119	540	0.7454	0.0307	599	0.1180	0.0070			
482	0.0983	0.0131	541	0.7336	0.0304	600	0.1138	0.0068			
483	0.1130	0.0143	542	0.7196	0.0300	601	0.1101	0.0065			
484	0.1280	0.0154	543	0.7102	0.0297	602	0.1067	0.0063			
485	0.1451	0.0167	544	0.7023	0.0295	603	0.1028	0.0061			
486	0.1627	0.0178	545	0.6957	0.0294	604	0.0996	0.0059			
487	0.1832	0.0190	546	0.6899	0.0292	605	0.0966	0.0057			
488	0.2042	0.0201	547	0.6847	0.0291	606	0.0935	0.0055			
489	0.2266	0.0212	548	0.6785	0.0289	607	0.0904	0.0054			
490	0.2515	0.0224	549	0.6724	0.0288	608	0.0874	0.0052			
491	0.2797	0.0238	550	0.6627	0.0285	609	0.0842	0.0051			
492	0.3085	0.0250	551	0.6531	0.0282	610	0.0809	0.0049			
493	0.3420	0.0265	552	0.6423	0.0279	611	0.0780	0.0048			
494	0.3780	0.0280	553	0.6293	0.0274	612	0.0747	0.0047			
495	0.4147	0.0294	554	0.6135	0.0269	613	0.0719	0.0046			
496	0.4568	0.0311	555	0.5961	0.0263	614	0.0686	0.0044			
497	0.5006	0.0328	556	0.5760	0.0257	615	0.0658	0.0043			
498	0.5445	0.0343	557	0.5553	0.0250	616	0.0630	0.0042			
499	0.5910	0.0360	558	0.5348	0.0243	617	0.0599	0.0040			
500	0.6362	0.0375	559	0.5129	0.0235	618	0.0567	0.0039			
501	0.6796	0.0388	560	0.4937	0.0229	619	0.0541	0.0038			
502	0.7211	0.0400	561	0.4725	0.0223	620	0.0515	0.0037			
503	0.7581	0.0409	562	0.4523	0.0216	621	0.0493	0.0035			
504	0.7880	0.0413	563	0.4333	0.0210	622	0.0467	0.0034			
505	0.8145	0.0417	564	0.4152	0.0205	623	0.0446	0.0033			
506	0.8349	0.0416	565	0.3985	0.0200	624	0.0427	0.0032			
507	0.8500	0.0414	566	0.3835	0.0196	625	0.0407	0.0031			
508	0.8592	0.0409	567	0.3704	0.0193	626	0.0390	0.0030			

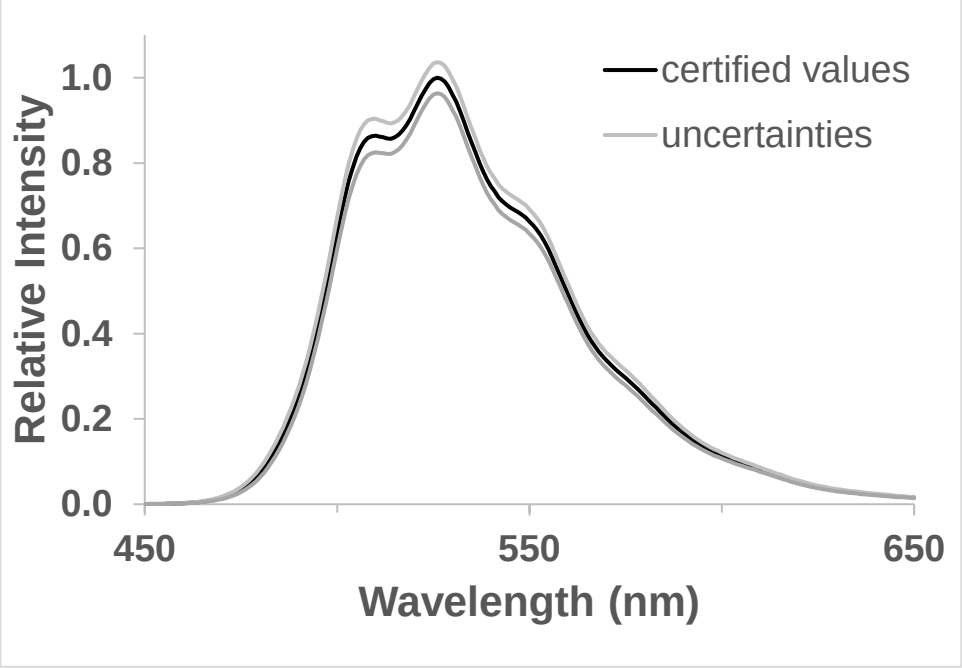

Figure 1. Spectrum for SRM 2941a Lot 1.

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 [1,2]. 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 2941a 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 2941a

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 2941a 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 emission monochromator was calibrated for wavelength using a Hg line of a pen lamp at 404.66 nm. The excitation monochromator was calibrated for wavelength by setting the excitation monochromator at 427.0 nm and then scanning the emission monochromator over the same wavelength position. A diffuse reflector was placed at the sample at a 45° angle relative to the incident beam to reflect the light from the excitation beam into the detection system. 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=2941a.

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 Oct 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 https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Oct 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 Oct 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).

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.