National Institute of Standards & Technology

Certificate

Standard Reference Material® 2943

Series Sample

Relative Intensity Correction Standard for Fluorescence Spectroscopy: Blue 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. SRM 2943 is a copper-doped (mole fraction of 0.01 % Cu_2O) phosphate-matrix glass. A unit of SRM 2943 consists of a single cuvette-shaped piece of solid glass. Each piece 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 17 units of SRM 2943 Series Sample with serial numbers xx through yy.

Certified Values: 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]. The certified values for the relative corrected emission spectrum (E) and corresponding combined 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. This SRM is certified for the relative, corrected emission spectrum, E, in relative energy units from emission wavelengths λ_{EM} = 350 nm to 640 nm at 1 nm wavelength intervals at a fixed excitation wavelength (λ_{EX}) of 330.3 nm. Due to larger signal-to-noise levels near the peak maximum, the emission range from λ_{EM} = 380 nm to 560 nm is recommended as optimal for most instruments and applications. 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.

Reference Values: This standard's certified values become reference values when used for spectral correction of fluorescence spectrometers with pulsed light sources. 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 associated uncertainty that may reflect only measurement precision and may not include all sources of uncertainty [1].

Expiration of Certification: The certification of **SRM 2943 Series Sample** 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 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.

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: 16 November 2023
Certificate Revision History on Last Page

Steven J. Choquette, Director
Office of Reference Materials

Table 1. Certified Relative Corrected Emission Spectrum of SRM 2943 Series Sample at λ_{EX} = 330.3 nm[(a)]

λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}
350	0.0121	0.0014	402	0.4351	0.0232	454	0.9801	0.0434	506	0.4419	0.0206
351	0.0129	0.0015	403	0.4523	0.0239	455	0.9754	0.0432	507	0.4308	0.0201
352	0.0137	0.0015	404	0.4733	0.0248	456	0.9716	0.0430	508	0.4209	0.0197
353	0.0150	0.0016	405	0.4920	0.0256	457	0.9660	0.0428	509	0.4100	0.0193
354	0.0161	0.0017	406	0.5119	0.0264	458	0.9594	0.0426	510	0.3993	0.0188
355	0.0173	0.0018	407	0.5301	0.0270	459	0.9555	0.0425	511	0.3895	0.0184
356	0.0188	0.0019	408	0.5499	0.0278	460	0.9452	0.0421	512	0.3797	0.0180
357	0.0202	0.0020	409	0.5684	0.0285	461	0.9397	0.0419	513	0.3694	0.0175
358	0.0220	0.0021	410	0.5849	0.0291	462	0.9305	0.0415	514	0.3602	0.0171
359	0.0234	0.0022	411	0.6030	0.0297	463	0.9232	0.0412	515	0.3510	0.0167
360	0.0259	0.0024	412	0.6219	0.0305	464	0.9126	0.0408	516	0.3418	0.0163
361	0.0282	0.0026	413	0.6417	0.0313	465	0.9035	0.0405	517	0.3327	0.0158
362	0.0308	0.0028	414	0.6587	0.0319	466	0.8940	0.0401	518	0.3241	0.0154
363	0.0337	0.0030	415	0.6757	0.0326	467	0.8821	0.0396	519	0.3154	0.0150
364	0.0367	0.0032	416	0.6932	0.0332	468	0.8704	0.0391	520	0.3070	0.0146
365	0.0403	0.0035	417	0.7116	0.0339	469	0.8612	0.0388	521	0.2987	0.0142
366	0.0436	0.0037	418	0.7248	0.0344	470	0.8494	0.0382	522	0.2914	0.0138
367	0.0472	0.0039	419	0.7416	0.0350	471	0.8395	0.0378	523	0.2831	0.0134
368	0.0514	0.0042	420	0.7550	0.0355	472	0.8287	0.0372	524	0.2752	0.0131
369	0.0553	0.0044	421	0.7731	0.0361	473	0.8192	0.0367	525	0.2676	0.0128
370	0.0601	0.0047	422	0.7887	0.0366	474	0.8081	0.0362	526	0.2599	0.0125
371	0.0655	0.0050	423	0.8036	0.0371	475	0.7961	0.0356	527	0.2536	0.0122
372	0.0705	0.0054	424	0.8188	0.0377	476	0.7859	0.0351	528	0.2467	0.0119
373	0.0768	0.0058	425	0.8315	0.0381	477	0.7727	0.0345	529	0.2400	0.0116
374	0.0828	0.0061	426	0.8426	0.0386	478	0.7607	0.0340	530	0.2334	0.0113
375	0.0893	0.0065	427	0.8560	0.0391	479	0.7480	0.0335	531	0.2279	0.0111
376	0.0961	0.0069	428	0.8670	0.0396	480	0.7368	0.0330	532	0.2218	0.0109
377	0.1042	0.0074	429	0.8762	0.0400	481	0.7262	0.0326	533	0.2162	0.0106
378	0.1110	0.0077	430	0.8880	0.0405	482	0.7155	0.0321	534	0.2105	0.0104
379	0.1192	0.0082	431	0.9037	0.0411	483	0.7046	0.0317	535	0.2053	0.0102
380	0.1266	0.0086	432	0.9126	0.0415	484	0.6945	0.0313	536	0.2000	0.0100
381	0.1370	0.0091	433	0.9240	0.0420	485	0.6825	0.0308	537	0.1951	0.0098
382	0.1462	0.0096	434	0.9347	0.0423	486	0.6701	0.0303	538	0.1901	0.0096
383	0.1575	0.0102	435	0.9478	0.0428	487	0.6587	0.0299	539	0.1853	0.0094
384	0.1675	0.0107	436	0.9556	0.0431	488	0.6476	0.0294	540	0.1804	0.0092
385	0.1783	0.0113	437	0.9639	0.0434	489	0.6356	0.0289	541	0.1760	0.0090
386	0.1908	0.0119	438	0.9693	0.0436	490	0.6246	0.0285	542	0.1713	0.0088
387	0.2032	0.0125	439	0.9789	0.0439	491	0.6141	0.0280	543	0.1668	0.0086
388	0.2152	0.0131	440	0.9838	0.0441	492	0.6015	0.0275	544	0.1624	0.0084
389	0.2273	0.0136	441	0.9899	0.0444	493	0.5893	0.0270	545	0.1581	0.0083
390	0.2391	0.0142	442	0.9901	0.0444	494	0.5762	0.0264	546	0.1541	0.0081
391	0.2536	0.0148	443	0.9930	0.0445	495	0.5660	0.0259	547	0.1501	0.0080
392	0.2681	0.0155	444	0.9976	0.0447	496	0.5536	0.0253	548	0.1460	0.0078
393	0.2836	0.0162	445	1.0000	0.0447	497	0.5415	0.0248	549	0.1420	0.0077
394	0.2999	0.0169	446	0.9995	0.0446	498	0.5305	0.0243	550	0.1382	0.0076
395	0.3159	0.0177	447	0.9985	0.0445	499	0.5190	0.0238	551	0.1346	0.0074
396	0.3319	0.0185	448	0.9962	0.0443	500	0.5063	0.0233	552	0.1313	0.0073
397	0.3478	0.0192	449	0.9920	0.0441	501	0.4967	0.0228	553	0.1277	0.0071
398	0.3651	0.0200	450	0.9920	0.0440	502	0.4857	0.0224	554	0.1242	0.0070
399	0.3811	0.0208	451	0.9898	0.0439	503	0.4738	0.0219	555	0.1209	0.0068
400	0.3984	0.0216	452	0.9874	0.0437	504	0.4636	0.0215	556	0.1175	0.0067
401	0.4171	0.0224	453	0.9829	0.0435	505	0.4531	0.0210	557	0.1142	0.0066

[(a)] Note that this standard's certified values become reference values when used for spectral correction of fluorescence spectrometers with pulsed light sources.

Table 1. Certified Relative Corrected Emission Spectrum of SRM 2943 Series Sample at λ_{EX} = 330.3 nm[(a)] (Continued)

λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}
558	0.1112	0.0065	579	0.0630	0.0051	600	0.0387	0.0045	621	0.0247	0.0039
559	0.1082	0.0064	580	0.0615	0.0050	601	0.0380	0.0044	622	0.0242	0.0039
560	0.1054	0.0063	581	0.0599	0.0050	602	0.0371	0.0044	623	0.0238	0.0038
561	0.1023	0.0062	582	0.0584	0.0050	603	0.0363	0.0044	624	0.0233	0.0038
562	0.0994	0.0061	583	0.0569	0.0049	604	0.0354	0.0044	625	0.0229	0.0038
563	0.0968	0.0061	584	0.0557	0.0049	605	0.0346	0.0043	626	0.0224	0.0038
564	0.0942	0.0060	585	0.0543	0.0049	606	0.0340	0.0043	627	0.0220	0.0037
565	0.0914	0.0059	586	0.0529	0.0048	607	0.0332	0.0043	628	0.0216	0.0037
566	0.0891	0.0058	587	0.0518	0.0048	608	0.0326	0.0043	629	0.0211	0.0037
567	0.0865	0.0057	588	0.0505	0.0048	609	0.0319	0.0043	630	0.0207	0.0037
568	0.0842	0.0056	589	0.0494	0.0047	610	0.0311	0.0042	631	0.0203	0.0037
569	0.0823	0.0056	590	0.0483	0.0047	611	0.0305	0.0042	632	0.0200	0.0037
570	0.0799	0.0055	591	0.0471	0.0047	612	0.0299	0.0042	633	0.0196	0.0036
571	0.0778	0.0054	592	0.0462	0.0047	613	0.0292	0.0042	634	0.0193	0.0036
572	0.0758	0.0054	593	0.0452	0.0046	614	0.0286	0.0041	635	0.0189	0.0036
573	0.0736	0.0053	594	0.0441	0.0046	615	0.0280	0.0041	636	0.0185	0.0036
574	0.0717	0.0053	595	0.0431	0.0046	616	0.0274	0.0041	637	0.0182	0.0036
575	0.0699	0.0053	596	0.0423	0.0046	617	0.0268	0.0040	638	0.0179	0.0035
576	0.0681	0.0052	597	0.0414	0.0046	618	0.0263	0.0040	639	0.0176	0.0035
577	0.0664	0.0052	598	0.0404	0.0045	619	0.0257	0.0040	640	0.0173	0.0035
578	0.0647	0.0051	599	0.0396	0.0045	620	0.0252	0.0039			

[(a)] Note that this standard's certified values become reference values when used for spectral correction of fluorescence spectrometers with pulsed light sources.

Information Values: A NIST information value is considered to be a value that will be of interest 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]. A NIST information value is provided for information purposes only. Information values are provided for the temperature coefficient of the E value at 445 nm and the fluorescence anisotropy (r) at 445 nm of SRM 2943 Series Sample are provided in Table 2. Information values cannot be used to establish metrological traceability.

Table 2: Temperature Coefficient of the E Value at 445 nm and the Fluorescence Anisotropy (r) at 445 nm of SRM 2943 Series Sample

Temperature Coefficient for E at 445 nm:	0.41 % $°C^{-1}$ (range: 11 °C to 39 °C)
Fluorescence Anisotropy (r) at 445 nm:	0.05

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.

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.

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 the area where the excitation beam will be incident 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.

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 330.3 nm. Scan the emission monochromator from 350 nm to 640 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 445 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=2943&cclcl=en_US.

For Use as a Day-to-Day Intensity Standard: Excite the SRM at a wavelength between 280 nm and 340 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 shape of the emission spectrum was observed within an uncertainty of ± 0.5 % (k=2) near the peak maximum. The absolute intensity decreased with time at a rate of 0.06 % per hour. This amount of irradiation corresponds to about 50 hours of irradiation with our fluorometer's excitation beam under the conditions used for certification. The relatively slow, but irreversible decrease in absolute intensity with irradiation time limits the use of this SRM as a day-to-day intensity standard.

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 350 nm to 640 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].

[(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.

The spectrum of each SRM was then collected from an excitation wavelength of 350 nm to 640 nm at 1 nm increments and a fixed excitation wavelength of 330.3 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 445 nm. The absolute peak intensity was 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 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 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 [1].

REFERENCES

[1] 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).

[2] DeRose, P.C.; Early, E.A.; Kramer, G.W.; *Qualification of a Fluorescence Spectrometer for Measuring True Fluorescence Spectra*; Rev. Sci. Intru., Vol. 78, p. 033107 (2007).

Certificate Revision History: 16 November 2023 (Change of expiration date; editorial changes); 02 May 2019 (Change of expiration date; editorial changes); 05 October 2009 (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.