National Institute of Standards & Technology

Certificate

Standard Reference Material® 2943

Sample Series

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 Sample Series with serial numbers Cu0xx 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 E and corresponding combined uncertainties at the 95 % confidence level, U_{95}, at each emission wavelength are given in Table 1. 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. Note that this standard's certified values become reference values when used for spectral correction of fluorescence spectrometers with pulsed light sources. 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: 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]. Note that this standard's certified values become reference values when used for spectral correction of fluorescence spectrometers with pulsed light sources. 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.

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 Sample Series.

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

Expiration of Certification: The certification of **SRM 2943 Sample Series** is valid, within the measurement uncertainties specified, until **01 December 2018**, 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.

The overall direction and coordination of the technical measurements required for certification of this SRM were performed by P.C. DeRose of the NIST Biochemical Science Division.

Laurie E. Locascio, Chief
Biochemical Science Division

Gaithersburg, MD 20899
Certificate Issue Date: 05 October 2009

Robert L. Watters, Jr., Chief
Measurement Services Division

Production and certification of this SRM were performed by P.C. DeRose, G.W. Kramer, and M.V. Smith of the NIST Biochemical Science Division and J.R. Anderson of the NIST Fabrication Technology Division. Assistance was provided by K.D. Mielenz of the NIST Optical Technology 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 Measurement Services Division.

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.

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 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. 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://www-s.nist.gov/srmors/view_detail.cfm?srm=2943.

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.

[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.

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].

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

λ_{EM} (nm)	E	U_{95}	λ_{EM} (nm)	E	U_{95}	λ_{EM} (nm)	E	U_{95}	λ_{EM} (nm)	E	U_{95}
350	0.0121	0.0014	409	0.5684	0.0285	468	0.8704	0.0391	527	0.2536	0.0122
351	0.0129	0.0015	410	0.5849	0.0291	469	0.8612	0.0388	528	0.2467	0.0119
352	0.0137	0.0015	411	0.6030	0.0297	470	0.8494	0.0382	529	0.2400	0.0116
353	0.0150	0.0016	412	0.6219	0.0305	471	0.8395	0.0378	530	0.2334	0.0113
354	0.0161	0.0017	413	0.6417	0.0313	472	0.8287	0.0372	531	0.2279	0.011
355	0.0173	0.0018	414	0.6587	0.0319	473	0.8192	0.0367	532	0.2218	0.011
356	0.0188	0.0019	415	0.6757	0.0326	474	0.8081	0.0362	533	0.2162	0.011
357	0.0202	0.0020	416	0.6932	0.0332	475	0.7961	0.0356	534	0.2105	0.010
358	0.0220	0.0021	417	0.7116	0.0339	476	0.7859	0.0351	535	0.2053	0.010
359	0.0234	0.0022	418	0.7248	0.0344	477	0.7727	0.0345	536	0.2000	0.010
360	0.0259	0.0024	419	0.7416	0.0350	478	0.7607	0.0340	537	0.1951	0.010
361	0.0282	0.0026	420	0.7550	0.0355	479	0.7480	0.0335	538	0.1901	0.010
362	0.0308	0.0028	421	0.7731	0.0361	480	0.7368	0.0330	539	0.1853	0.009
363	0.0337	0.0030	422	0.7887	0.0366	481	0.7262	0.0326	540	0.1804	0.009
364	0.0367	0.0032	423	0.8036	0.0371	482	0.7155	0.0321	541	0.1760	0.009
365	0.0403	0.0035	424	0.8188	0.0377	483	0.7046	0.0317	542	0.1713	0.009
366	0.0436	0.0037	425	0.8315	0.0381	484	0.6945	0.0313	543	0.1668	0.009
367	0.0472	0.0039	426	0.8426	0.0386	485	0.6825	0.0308	544	0.1624	0.008
368	0.0514	0.0042	427	0.8560	0.0391	486	0.6701	0.0303	545	0.1581	0.008
369	0.0553	0.0044	428	0.8670	0.0396	487	0.6587	0.0299	546	0.1541	0.008
370	0.0601	0.0047	429	0.8762	0.0400	488	0.6476	0.0294	547	0.1501	0.008
371	0.0655	0.0050	430	0.8880	0.0405	489	0.6356	0.0289	548	0.1460	0.008
372	0.0705	0.0054	431	0.9037	0.0411	490	0.6246	0.0285	549	0.1420	0.008
373	0.0768	0.0058	432	0.9126	0.0415	491	0.6141	0.0280	550	0.1382	0.008
374	0.0828	0.0061	433	0.9240	0.0420	492	0.6015	0.0275	551	0.1346	0.007
375	0.0893	0.0065	434	0.9347	0.0423	493	0.5893	0.0270	552	0.1313	0.007
376	0.0961	0.0069	435	0.9478	0.0428	494	0.5762	0.0264	553	0.1277	0.007
377	0.1042	0.0074	436	0.9556	0.0431	495	0.5660	0.0259	554	0.1242	0.007
378	0.1110	0.0077	437	0.9639	0.0434	496	0.5536	0.0253	555	0.1209	0.007
379	0.1192	0.0082	438	0.9693	0.0436	497	0.5415	0.0248	556	0.1175	0.007
380	0.1266	0.0086	439	0.9789	0.0439	498	0.5305	0.0243	557	0.1142	0.007
381	0.1370	0.0091	440	0.9838	0.0441	499	0.5190	0.0238	558	0.1112	0.007
382	0.1462	0.0096	441	0.9899	0.0444	500	0.5063	0.0233	559	0.1082	0.006
383	0.1575	0.0102	442	0.9901	0.0444	501	0.4967	0.0228	560	0.1054	0.006
384	0.1675	0.0107	443	0.9930	0.0445	502	0.4857	0.0224	561	0.1023	0.006
385	0.1783	0.0113	444	0.9976	0.0447	503	0.4738	0.0219	562	0.0994	0.006
386	0.1908	0.0119	445	1.0000	0.0447	504	0.4636	0.0215	563	0.0968	0.006
387	0.2032	0.0125	446	0.9995	0.0446	505	0.4531	0.0210	564	0.0942	0.006
388	0.2152	0.0131	447	0.9985	0.0445	506	0.4419	0.0206	565	0.0914	0.006
389	0.2273	0.0136	448	0.9962	0.0443	507	0.4308	0.0201	566	0.0891	0.006
390	0.2391	0.0142	449	0.9920	0.0441	508	0.4209	0.0197	567	0.0865	0.006
391	0.2536	0.0148	450	0.9920	0.0440	509	0.4100	0.0193	568	0.0842	0.006
392	0.2681	0.0155	451	0.9898	0.0439	510	0.3993	0.0188	569	0.0823	0.006
393	0.2836	0.0162	452	0.9874	0.0437	511	0.3895	0.0184	570	0.0799	0.006
394	0.2999	0.0169	453	0.9829	0.0435	512	0.3797	0.0180	571	0.0778	0.005
395	0.3159	0.0177	454	0.9801	0.0434	513	0.3694	0.0175	572	0.0758	0.005
396	0.3319	0.0185	455	0.9754	0.0432	514	0.3602	0.0171	573	0.0736	0.005
397	0.3478	0.0192	456	0.9716	0.0430	515	0.3510	0.0167	574	0.0717	0.005
398	0.3651	0.0200	457	0.9660	0.0428	516	0.3418	0.0163	575	0.0699	0.005
399	0.3811	0.0208	458	0.9594	0.0426	517	0.3327	0.0158	576	0.0681	0.005
400	0.3984	0.0216	459	0.9555	0.0425	518	0.3241	0.0154	577	0.0664	0.005
401	0.4171	0.0224	460	0.9452	0.0421	519	0.3154	0.0150	578	0.0647	0.005
402	0.4351	0.0232	461	0.9397	0.0419	520	0.3070	0.0146	579	0.0630	0.005
403	0.4523	0.0239	462	0.9305	0.0415	521	0.2987	0.0142	580	0.0615	0.005
404	0.4733	0.0248	463	0.9232	0.0412	522	0.2914	0.0138	581	0.0599	0.005
405	0.4920	0.0256	464	0.9126	0.0408	523	0.2831	0.0134	582	0.0584	0.005
406	0.5119	0.0264	465	0.9035	0.0405	524	0.2752	0.0131	583	0.0569	0.005
407	0.5301	0.0270	466	0.8940	0.0401	525	0.2676	0.0128	584	0.0557	0.005

λ_{EM} (nm)	E	U_{95}	λ_{EM} (nm)	E	U_{95}	λ_{EM} (nm)	E	U_{95}	λ_{EM} (nm)	E	U_{95}
408	0.5499	0.0278	467	0.8821	0.0396	526	0.2599	0.0125	585	0.0543	0.005
586	0.0529	0.005	600	0.0387	0.004	614	0.0286	0.004	628	0.0216	0.004
587	0.0518	0.005	601	0.0380	0.004	615	0.0280	0.004	629	0.0211	0.004
588	0.0505	0.005	602	0.0371	0.004	616	0.0274	0.004	630	0.0207	0.004
589	0.0494	0.005	603	0.0363	0.004	617	0.0268	0.004	631	0.0203	0.004
590	0.0483	0.005	604	0.0354	0.004	618	0.0263	0.004	632	0.0200	0.004
591	0.0471	0.005	605	0.0346	0.004	619	0.0257	0.004	633	0.0196	0.004
592	0.0462	0.005	606	0.0340	0.004	620	0.0252	0.004	634	0.0193	0.004
593	0.0452	0.005	607	0.0332	0.004	621	0.0247	0.004	635	0.0189	0.004
594	0.0441	0.005	608	0.0326	0.004	622	0.0242	0.004	636	0.0185	0.004
595	0.0431	0.005	609	0.0319	0.004	623	0.0238	0.004	637	0.0182	0.004
596	0.0423	0.005	610	0.0311	0.004	624	0.0233	0.004	638	0.0179	0.004
597	0.0414	0.005	611	0.0305	0.004	625	0.0229	0.004	639	0.0176	0.004
598	0.0404	0.005	612	0.0299	0.004	626	0.0224	0.004	640	0.0173	0.003
599	0.0396	0.004	613	0.0292	0.004	627	0.0220	0.004			

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

REFERENCES

[1] JCGM 100:2008; *Guide to the Expression of Uncertainty in Measurement;* (ISO GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology: BIPM, Sevres Cedex, France (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf; 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://www.physics.nist.gov/Pubs/contents.html.

[2] DeRose, P.C.; Early, E.A.; Kramer, G.W.; *Qualification of a fluorescence spectrometer for measuring true fluorescence spectra*; Rev. Sci. Intru., 78, 033107 (2007).

[3] ISO; *Guide 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/.

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