National Institute of Standards & Technology

Certificate

Standard Reference Material® 2944

Relative Intensity Correction Standard for Fluorescence Spectroscopy: Red Emission

Sample Series

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. A unit of SRM 2944 consists of a single cuvette-shaped piece of solid glass. Sample Series units have a serial number between Bi0XX and Bi0YY. This SRM is certified for the relative, corrected emission spectrum, E, in relative energy units from emission wavelengths λ_{EM} = 530 nm to 830 nm at 1 nm wavelength intervals at a fixed excitation wavelength (λ_{EX}) of 515.0 nm. Due to larger signal to noise levels near the peak maximum, the emission range from λ_{EM} = 590 nm to 805 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.

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 total uncertainties at the 95 % confidence level, U_{95}, at each emission wavelength are given in Table 1 and Table 2. 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.

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. The values in Table 1 and Table 2 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.

Expiration of Certification: The certification of **SRM 2944** is valid, within the measurement uncertainty specified, until **31 August 2025**, provided the SRM is handled and stored in accordance with instructions given in this certificate (see "Instructions for Handling, Storage, and 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 technical 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.

Production and certification of this SRM were performed by P.C. DeRose of the NIST Biosystems and Biomaterials Division, M.V. Smith of the NIST Chemical Sciences Division, and G.W. Kramer and J.R. Anderson formerly of NIST.

Overall direction and coordination of the technical measurements required for certification of this SRM were performed by P.C. DeRose.

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: 22 July 2020
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Table 1. Relative Corrected Emission Spectrum of SRM 2944 Sample Series at λ_{EX} = 515.0 nm

λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}
530	0.0020	0.0002	589	0.0888	0.0047	648	0.5952	0.0264	707	0.9937	0.0414
531	0.0021	0.0002	590	0.0930	0.0049	649	0.6060	0.0268	708	0.9926	0.0413
532	0.0022	0.0002	591	0.0973	0.0051	650	0.6179	0.0273	709	0.9899	0.0412
533	0.0024	0.0002	592	0.1015	0.0053	651	0.6285	0.0277	710	0.9870	0.0411
534	0.0026	0.0002	593	0.1062	0.0055	652	0.6409	0.0282	711	0.9842	0.0410
535	0.0027	0.0002	594	0.1108	0.0057	653	0.6507	0.0286	712	0.9813	0.0410
536	0.0029	0.0002	595	0.1157	0.0059	654	0.6630	0.0290	713	0.9803	0.0409
537	0.0031	0.0003	596	0.1208	0.0062	655	0.6735	0.0294	714	0.9769	0.0408
538	0.0034	0.0003	597	0.1257	0.0064	656	0.6868	0.0299	715	0.9731	0.0407
539	0.0036	0.0003	598	0.1309	0.0067	657	0.6971	0.0303	716	0.9692	0.0406
540	0.0039	0.0003	599	0.1361	0.0069	658	0.7086	0.0307	717	0.9619	0.0404
541	0.0042	0.0003	600	0.1418	0.0072	659	0.7196	0.0311	718	0.9576	0.0402
542	0.0046	0.0004	601	0.1477	0.0074	660	0.7298	0.0315	719	0.9515	0.0400
543	0.0050	0.0004	602	0.1539	0.0077	661	0.7413	0.0319	720	0.9470	0.0399
544	0.0054	0.0004	603	0.1601	0.0080	662	0.7529	0.0323	721	0.9397	0.0396
545	0.0059	0.0005	604	0.1664	0.0083	663	0.7642	0.0327	722	0.9288	0.0393
546	0.0064	0.0005	605	0.1729	0.0085	664	0.7745	0.0331	723	0.9209	0.0390
547	0.0069	0.0005	606	0.1796	0.0088	665	0.7857	0.0335	724	0.9119	0.0387
548	0.0075	0.0005	607	0.1866	0.0092	666	0.7957	0.0339	725	0.9028	0.0384
549	0.0081	0.0006	608	0.1936	0.0095	667	0.8056	0.0343	726	0.8936	0.0380
550	0.0086	0.0006	609	0.2008	0.0098	668	0.8162	0.0347	727	0.8838	0.0377
551	0.0092	0.0007	610	0.2081	0.0102	669	0.8267	0.0350	728	0.8724	0.0373
552	0.0098	0.0007	611	0.2162	0.0105	670	0.8360	0.0354	729	0.8614	0.0370
553	0.0104	0.0007	612	0.2237	0.0109	671	0.8468	0.0358	730	0.8514	0.0366
554	0.0111	0.0008	613	0.2319	0.0112	672	0.8563	0.0361	731	0.8398	0.0362
555	0.0119	0.0008	614	0.2399	0.0116	673	0.8651	0.0364	732	0.8294	0.0359
556	0.0127	0.0009	615	0.2479	0.0119	674	0.8747	0.0367	733	0.8182	0.0355
557	0.0136	0.0009	616	0.2567	0.0123	675	0.8837	0.0370	734	0.8087	0.0351
558	0.0146	0.0010	617	0.2655	0.0127	676	0.8900	0.0373	735	0.7963	0.0347
559	0.0157	0.0010	618	0.2750	0.0131	677	0.8980	0.0375	736	0.7852	0.0343
560	0.0167	0.0011	619	0.2835	0.0135	678	0.9060	0.0379	737	0.7733	0.0339
561	0.0179	0.0012	620	0.2929	0.0139	679	0.9131	0.0382	738	0.7609	0.0335
562	0.0192	0.0012	621	0.3022	0.0143	680	0.9196	0.0384	739	0.7483	0.0330
563	0.0206	0.0013	622	0.3122	0.0147	681	0.9271	0.0387	740	0.7372	0.0327
564	0.0220	0.0014	623	0.3228	0.0151	682	0.9334	0.0390	741	0.7248	0.0322
565	0.0235	0.0015	624	0.3326	0.0156	683	0.9400	0.0393	742	0.7112	0.0317
566	0.0250	0.0015	625	0.3427	0.0160	684	0.9466	0.0395	743	0.6985	0.0313
567	0.0267	0.0016	626	0.3535	0.0165	685	0.9521	0.0397	744	0.6882	0.0309
568	0.0284	0.0017	627	0.3642	0.0170	686	0.9580	0.0399	745	0.6755	0.0304
569	0.0302	0.0018	628	0.3750	0.0174	687	0.9625	0.0401	746	0.6628	0.0299
570	0.0322	0.0019	629	0.3860	0.0179	688	0.9662	0.0402	747	0.6497	0.0294
571	0.0342	0.0020	630	0.3972	0.0184	689	0.9699	0.0404	748	0.6369	0.0289
572	0.0363	0.0021	631	0.4072	0.0188	690	0.9740	0.0405	749	0.6251	0.0285
573	0.0386	0.0022	632	0.4185	0.0193	691	0.9773	0.0406	750	0.6122	0.0280
574	0.0409	0.0023	633	0.4291	0.0197	692	0.9805	0.0407	751	0.6004	0.0275
575	0.0433	0.0025	634	0.4400	0.0202	693	0.9842	0.0409	752	0.5855	0.0270
576	0.0459	0.0026	635	0.4515	0.0206	694	0.9875	0.0410	753	0.5727	0.0265
577	0.0486	0.0027	636	0.4619	0.0211	695	0.9894	0.0411	754	0.5600	0.0260
578	0.0513	0.0029	637	0.4727	0.0215	696	0.9931	0.0412	755	0.5483	0.0255
579	0.0542	0.0030	638	0.4837	0.0219	697	0.9936	0.0413	756	0.5352	0.0249
580	0.0570	0.0032	639	0.4949	0.0224	698	0.9945	0.0414	757	0.5222	0.0244
581	0.0603	0.0033	640	0.5059	0.0228	699	0.9952	0.0414	758	0.5087	0.0238
582	0.0636	0.0035	641	0.5175	0.0233	700	0.9985	0.0415	759	0.4975	0.0233
583	0.0669	0.0036	642	0.5287	0.0237	701	0.9984	0.0416	760	0.4850	0.0228
584	0.0702	0.0038	643	0.5397	0.0242	702	0.9978	0.0416	761	0.4710	0.0222
585	0.0736	0.0040	644	0.5499	0.0246	703	0.9983	0.0416	762	0.4591	0.0217
586	0.0774	0.0041	645	0.5612	0.0250	704	1.0000	0.0416	763	0.4476	0.0212
587	0.0810	0.0043	646	0.5726	0.0255	705	0.9997	0.0416	764	0.4363	0.0207
588	0.0848	0.0045	647	0.5832	0.0259	706	0.9954	0.0415	765	0.4241	0.0202

Table 2. Relative Corrected Emission Spectrum of SRM 2944 Sample Series at λ_{EX} = 515.0 nm

λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}
766	0.4122	0.0197	783	0.2341	0.0118	800	0.1290	0.0069	817	0.0680	0.0058
767	0.3994	0.0191	784	0.2260	0.0114	801	0.1243	0.0067	818	0.0652	0.0058
768	0.3866	0.0186	785	0.2181	0.0110	802	0.1193	0.0065	819	0.0630	0.0060
769	0.3759	0.0181	786	0.2099	0.0106	803	0.1152	0.0063	820	0.0606	0.0060
770	0.3654	0.0176	787	0.2027	0.0103	804	0.1111	0.0062	821	0.0588	0.0062
771	0.3545	0.0172	788	0.1965	0.0100	805	0.1073	0.0060	822	0.0567	0.0063
772	0.3425	0.0167	789	0.1899	0.0097	806	0.1034	0.0059	823	0.0549	0.0065
773	0.3309	0.0161	790	0.1834	0.0094	807	0.0996	0.0058	824	0.0528	0.0066
774	0.3195	0.0156	791	0.1771	0.0091	808	0.0960	0.0057	825	0.0505	0.0067
775	0.3086	0.0152	792	0.1714	0.0089	809	0.0924	0.0057	826	0.0482	0.0067
776	0.2995	0.0147	793	0.1652	0.0086	810	0.0889	0.0056	827	0.0470	0.0067
777	0.2896	0.0143	794	0.1592	0.0084	811	0.0857	0.0056	828	0.0462	0.0068
778	0.2792	0.0139	795	0.1541	0.0081	812	0.0823	0.0056	829	0.0449	0.0069
779	0.2697	0.0134	796	0.1489	0.0079	813	0.0793	0.0055	830	0.0433	0.0070
780	0.2603	0.0130	797	0.1434	0.0077	814	0.0767	0.0056			
781	0.2514	0.0126	798	0.1383	0.0074	815	0.0738	0.0056			
782	0.2427	0.0122	799	0.1335	0.0072	816	0.0710	0.0057			

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]. Certified values for SRM 2944 become reference values when the unit is not positioned as previously indicated.

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]. A NIST information value is provided for information purposes only. Information values are provided for the temperature coefficient of the E value at 704 nm and the fluorescence anisotropy (r) at 704 nm of SRM 2944 Sample Series. Information values cannot be used to establish metrological traceability.

Temperature Coefficient (E at 704 nm) = –0.25 %•°C^{-1} (range: 11 °C to 39 °C) r (704 nm) = 0.10

Physical Description: SRM 2944 is a bismuth-doped (0.11 % Bi_2O_3 mole fraction) phosphate 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 for more than 30 hours with a white light source having a nominal intensity of 0.37 mW•cm^{-2}•nm^{-1} from 400 nm to 680 nm, no change in the absolute intensity or shape of the emission spectrum was observed within an uncertainty of ± 0.5 % (k = 2) at the peak maximum. This amount of irradiation corresponds to about 540 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 from 530 nm to 830 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 530 nm to 830 nm at 1 nm increments and a fixed excitation wavelength of 515.0 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 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 704 nm. The absolute peak intensity was also found to vary by 6 % or less for all units in this batch.

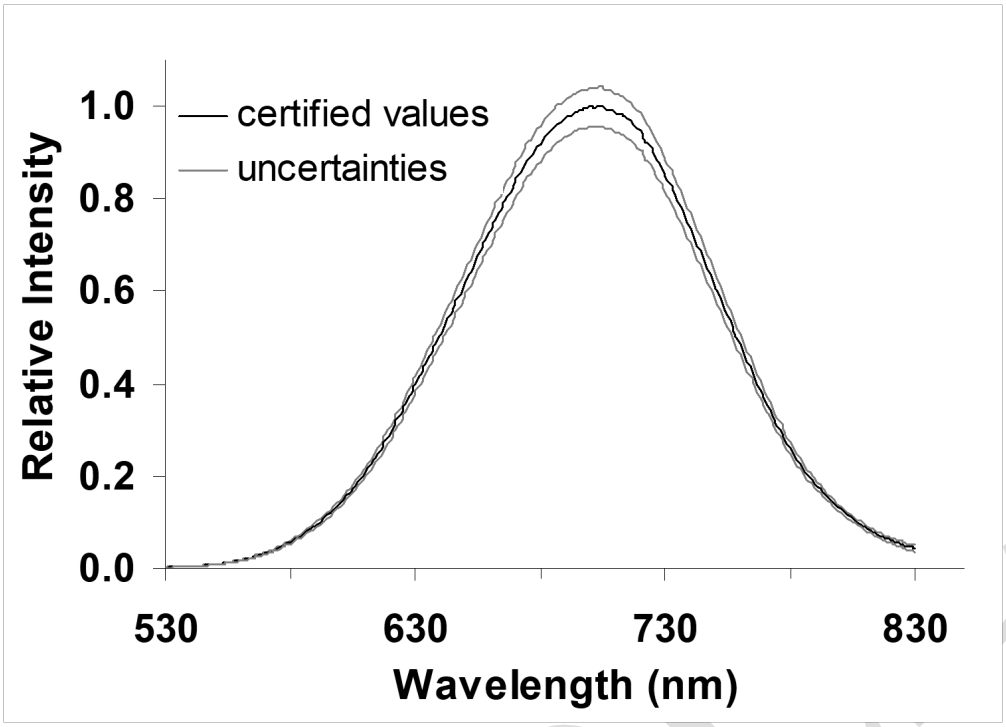

Figure 1. Certified Spectrum for SRM 2944 Sample Series.

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 [3].

INSTRUCTIONS FOR HANDLING, STORAGE, AND USE

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 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 400 nm as this will cause significant photodegradation.

For Use As a Day-to-Day Intensity Standard: Excite the SRM at a wavelength between 400 nm and 680 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.

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 515.0 nm. Scan the emission monochromator from 530 nm to 830 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 704 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 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_datafiles.cfm?srm=2944.

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.; *Definition of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260-136 (2000); U.S. Government Printing Office: Washington, DC (2000); available at https://www.nist.gov/system/files/documents/srm/SP260-136.PDF (accessed Jul 2020).

[2] DeRose, P.C.; Early, E.A.; Kramer, G.W.; *Qualification of a Fluorescence Spectrometer for Measuring True Fluorescence Spectra*; Rev. Sci. Instrum., Vol. 78, pp. 033107-1–033107-12 (2007).

[3] JCGM 100:2008; *Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement* (ISO GUM 1995 with Minor Corrections); Joint Committee for Guides in Metrology (2008); available at https://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Jul 2020); 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 Jul 2020).

Certificate Revision History: 22 July 2020 (Change of expiration date; editorial changes); **06 September 2011** (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: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at https://www.nist.gov/srm.