National Institute of Standards & Technology

Certificate

Standard Reference Material® 2940

Relative Intensity Correction Standard for Fluorescence Spectroscopy: Orange Emission

Series Sample

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 energy units from emission wavelengths λ_{EM} = 500nm to 800 nm at 1 nm wavelength intervals at a fixed excitation wavelength (λ_{EX}) of 412 nm. 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.

Expiration of Certification: The certification of **SRM 2940** is valid, within the measurement uncertainty specified, until **01 January 2023**, 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 G.W. Kramer formerly of NIST.

Production and certification of this SRM were performed by P.C. DeRose, M.V. Smith, and D.H. Blackburn of the NIST Biosystems and Biomaterials Division. Assistance was provided by E.A. Early and K.D. Mielenz formerly of NIST, and D.L. Duewer of the NIST Chemical Sciences 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 Office of Reference Materials.

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Certificate Revision History on Last Page

Steven J. Choquette, Director
Office of Reference Materials

Table 1. Certified Relative Corrected Emission Spectrum of SRM 2940 Series Sample at λ_{EX} = 412 nm

λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}
500	0.027	0.004	559	0.485	0.030	618	0.999	0.041	677	0.664	0.034
501	0.029	0.005	560	0.497	0.031	619	0.998	0.040	678	0.654	0.033
502	0.032	0.005	561	0.509	0.031	620	1.000	0.040	679	0.645	0.033
503	0.034	0.005	562	0.523	0.032	621	1.000	0.041	680	0.635	0.032
504	0.037	0.006	563	0.536	0.032	622	0.998	0.041	681	0.627	0.033
505	0.040	0.006	564	0.548	0.032	623	0.996	0.042	682	0.620	0.032
506	0.044	0.007	565	0.561	0.032	624	0.997	0.040	683	0.613	0.031
507	0.047	0.007	566	0.574	0.033	625	0.996	0.042	684	0.603	0.031
508	0.051	0.007	567	0.587	0.033	626	0.995	0.039	685	0.595	0.031
509	0.054	0.008	568	0.600	0.033	627	0.993	0.041	686	0.587	0.031
510	0.058	0.008	569	0.613	0.034	628	0.990	0.041	687	0.578	0.030
511	0.063	0.009	570	0.625	0.034	629	0.987	0.041	688	0.570	0.030
512	0.067	0.009	571	0.638	0.034	630	0.985	0.041	689	0.562	0.030
513	0.072	0.010	572	0.652	0.035	631	0.982	0.042	690	0.553	0.029
514	0.077	0.010	573	0.664	0.035	632	0.979	0.042	691	0.544	0.029
515	0.081	0.011	574	0.677	0.035	633	0.975	0.040	692	0.536	0.029
516	0.086	0.011	575	0.689	0.035	634	0.970	0.042	693	0.527	0.028
517	0.092	0.012	576	0.702	0.035	635	0.967	0.041	694	0.520	0.028
518	0.097	0.012	577	0.715	0.037	636	0.961	0.041	695	0.511	0.029
519	0.103	0.013	578	0.727	0.036	637	0.959	0.041	696	0.503	0.028
520	0.109	0.014	579	0.739	0.036	638	0.954	0.041	697	0.494	0.028
521	0.116	0.014	580	0.751	0.036	639	0.948	0.041	698	0.485	0.027
522	0.122	0.015	581	0.763	0.036	640	0.942	0.041	699	0.478	0.027
523	0.129	0.015	582	0.774	0.036	641	0.938	0.040	700	0.470	0.026
524	0.136	0.016	583	0.784	0.037	642	0.932	0.040	701	0.463	0.026
525	0.143	0.017	584	0.794	0.038	643	0.926	0.040	702	0.455	0.026
526	0.150	0.017	585	0.805	0.037	644	0.920	0.041	703	0.448	0.026
527	0.158	0.018	586	0.816	0.037	645	0.916	0.040	704	0.440	0.026
528	0.166	0.018	587	0.825	0.038	646	0.909	0.041	705	0.433	0.025
529	0.173	0.019	588	0.834	0.037	647	0.903	0.039	706	0.426	0.025
530	0.182	0.020	589	0.845	0.038	648	0.897	0.038	707	0.420	0.024
531	0.190	0.020	590	0.855	0.038	649	0.891	0.039	708	0.413	0.025
532	0.198	0.021	591	0.863	0.038	650	0.886	0.039	709	0.406	0.022
533	0.206	0.021	592	0.871	0.038	651	0.879	0.039	710	0.399	0.024
534	0.215	0.022	593	0.879	0.038	652	0.873	0.039	711	0.392	0.024
535	0.224	0.022	594	0.887	0.038	653	0.866	0.038	712	0.385	0.023
536	0.233	0.023	595	0.895	0.038	654	0.860	0.039	713	0.378	0.022
537	0.243	0.023	596	0.904	0.038	655	0.850	0.038	714	0.371	0.022
538	0.252	0.024	597	0.911	0.040	656	0.844	0.040	715	0.364	0.022
539	0.262	0.024	598	0.916	0.039	657	0.836	0.039	716	0.359	0.021
540	0.272	0.025	599	0.924	0.040	658	0.828	0.038	717	0.352	0.021
541	0.282	0.025	600	0.930	0.040	659	0.819	0.039	718	0.345	0.021
542	0.291	0.026	601	0.936	0.039	660	0.812	0.038	719	0.339	0.021
543	0.302	0.026	602	0.944	0.039	661	0.805	0.038	720	0.333	0.020
544	0.312	0.027	603	0.948	0.039	662	0.798	0.037	721	0.327	0.020
545	0.322	0.027	604	0.954	0.040	663	0.790	0.038	722	0.320	0.019
546	0.333	0.028	605	0.960	0.039	664	0.781	0.038	723	0.314	0.020
547	0.344	0.029	606	0.965	0.041	665	0.770	0.037	724	0.309	0.019
548	0.355	0.029	607	0.969	0.040	666	0.762	0.036	725	0.303	0.019
549	0.366	0.029	608	0.973	0.039	667	0.753	0.037	726	0.297	0.019
550	0.377	0.029	609	0.977	0.040	668	0.745	0.036	727	0.291	0.018
551	0.389	0.030	610	0.980	0.039	669	0.736	0.035	728	0.285	0.018
552	0.400	0.030	611	0.983	0.040	670	0.727	0.035	729	0.280	0.018
553	0.412	0.031	612	0.987	0.041	671	0.717	0.035	730	0.275	0.017
554	0.424	0.031	613	0.991	0.040	672	0.708	0.036	731	0.270	0.018
555	0.435	0.031	614	0.993	0.041	673	0.699	0.034	732	0.265	0.017
556	0.448	0.032	615	0.995	0.041	674	0.689	0.034	733	0.259	0.016
557	0.460	0.032	616	0.998	0.040	675	0.681	0.035	734	0.254	0.017
558	0.473	0.033	617	0.998	0.041	676	0.672	0.034	735	0.249	0.017

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Table 1 Continued

λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}
736	0.244	0.016	753	0.175	0.013	770	0.124	0.010	787	0.088	0.007
737	0.240	0.016	754	0.172	0.014	771	0.121	0.010	788	0.086	0.008
738	0.235	0.016	755	0.168	0.012	772	0.119	0.010	789	0.084	0.008
739	0.231	0.016	756	0.165	0.012	773	0.116	0.010	790	0.082	0.008
740	0.226	0.015	757	0.162	0.013	774	0.114	0.010	791	0.081	0.008
741	0.222	0.015	758	0.159	0.012	775	0.113	0.009	792	0.078	0.008
742	0.218	0.015	759	0.156	0.012	776	0.110	0.010	793	0.077	0.007
743	0.213	0.015	760	0.153	0.011	777	0.108	0.009	794	0.076	0.007
744	0.209	0.014	761	0.150	0.011	778	0.106	0.010	795	0.074	0.007
745	0.205	0.015	762	0.147	0.012	779	0.104	0.009	796	0.073	0.007
746	0.201	0.014	763	0.144	0.012	780	0.102	0.010	797	0.071	0.008
747	0.197	0.013	764	0.142	0.011	781	0.099	0.009	798	0.069	0.008
748	0.193	0.014	765	0.138	0.010	782	0.097	0.009	799	0.068	0.007
749	0.189	0.014	766	0.135	0.011	783	0.095	0.008	800	0.067	0.007
750	0.185	0.014	767	0.132	0.010	784	0.093	0.009			
751	0.181	0.014	768	0.130	0.010	785	0.091	0.008			
752	0.178	0.014	769	0.127	0.011	786	0.089	0.009			

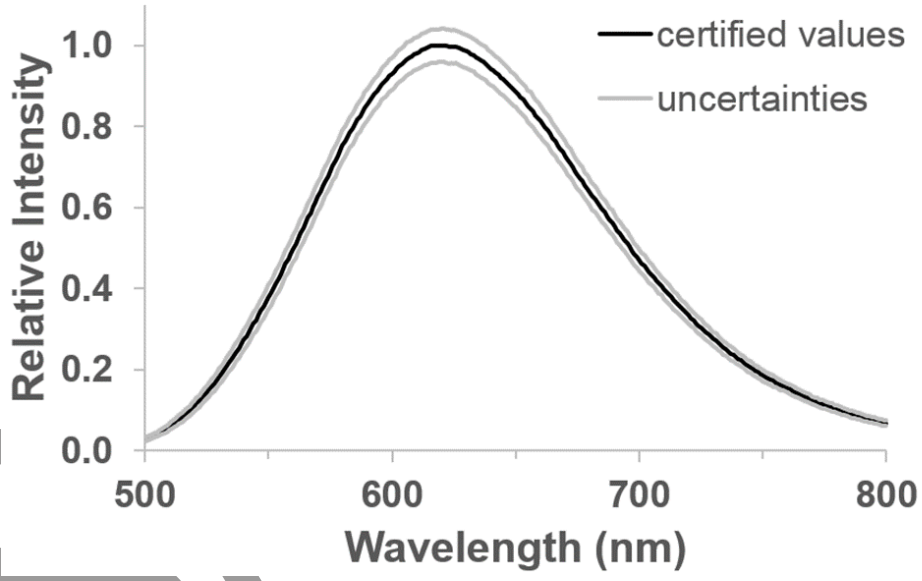

Figure 1. Certified Spectrum for SRM 2940 Sample Series.

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 620 nm and the fluorescence anisotropy (r) at 620 nm of SRM 2940 are listed in Table 2. Information values cannot be used to establish metrological traceability.

Table 2: Temperature Coefficient of the E Value at 620 nm and the Fluorescence Anisotropy at 620 nm of SRM 2940

Relative Temperature Coefficient for E (at 620 nm):	–0.21 % $°C^{-1}$ (range: 11 °C to 39 °C)
Fluorescence Anisotropy at 620 nm:	0.021

Physical Description: SRM 2940 is a manganese-doped (0.11 % MnO_2 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 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 500 nm to 800 nm at 1 nm increments and a fixed excitation wavelength of 412 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 620 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

WARNING TO USERS: This standard and its certified values cannot be used for spectral correction of fluorescence spectrometers with pulsed light sources. The certified values cannot be applied to instruments using pulsed light sources.

For Correction of Detection System Responsivity: Place 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 412 nm. Scan the emission monochromator from 500 nm to 800 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 620 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=2940.

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For Day-to-Day Intensity Standard: Excite the SRM at a wavelength between 400 nm and 550 nm, preferably at 412 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 May 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 http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed May 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 May 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).

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Certificate Revision History: 01 May 2018 (Change of expiration date, increase in the corresponding uncertainty values; addition of Figure 1; editorial changes); 18 April 2007 (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 https://www.nist.gov/srm.