



National Institute of Standards & Technology

Certificate

Standard Reference Material[®] 2940a

Relative Intensity Correction Standard for Fluorescence Spectroscopy: Orange Emission

Lot 3

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 $\lambda_{EM} = 500\text{ nm}$ 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 $25.0^\circ\text{C} \pm 0.5^\circ\text{C}$ 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 2940a** 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.

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Table 1. Certified Relative Corrected Emission Spectrum of SRM 2940a Lot 3 at $\lambda_{\text{EX}} = 412 \text{ nm}$

λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}
500	0.0326	0.0051	559	0.5285	0.0326	618	0.9991	0.0370	677	0.6314	0.0276
501	0.0353	0.0054	560	0.5406	0.0328	619	0.9983	0.0370	678	0.6230	0.0273
502	0.0385	0.0059	561	0.5528	0.0329	620	0.9965	0.0369	679	0.6157	0.0271
503	0.0422	0.0063	562	0.5668	0.0332	621	0.9962	0.0370	680	0.6066	0.0268
504	0.0453	0.0067	563	0.5804	0.0335	622	0.9925	0.0369	681	0.5973	0.0265
505	0.0487	0.0071	564	0.5909	0.0335	623	0.9908	0.0368	682	0.5923	0.0264
506	0.0530	0.0076	565	0.6045	0.0338	624	0.9857	0.0367	683	0.5819	0.0260
507	0.0569	0.0081	566	0.6187	0.0340	625	0.9837	0.0367	684	0.5748	0.0258
508	0.0612	0.0086	567	0.6318	0.0342	626	0.9800	0.0366	685	0.5671	0.0256
509	0.0660	0.0091	568	0.6437	0.0343	627	0.9770	0.0365	686	0.5588	0.0253
510	0.0705	0.0096	569	0.6557	0.0344	628	0.9760	0.0366	687	0.5544	0.0252
511	0.0752	0.0101	570	0.6687	0.0346	629	0.9720	0.0365	688	0.5440	0.0248
512	0.0807	0.0107	571	0.6829	0.0348	630	0.9684	0.0364	689	0.5361	0.0246
513	0.0861	0.0113	572	0.6949	0.0349	631	0.9639	0.0363	690	0.5285	0.0243
514	0.0917	0.0118	573	0.7080	0.0351	632	0.9594	0.0362	691	0.5217	0.0241
515	0.0974	0.0124	574	0.7190	0.0351	633	0.9558	0.0362	692	0.5141	0.0238
516	0.1036	0.0130	575	0.7318	0.0353	634	0.9498	0.0360	693	0.5052	0.0235
517	0.1102	0.0136	576	0.7444	0.0354	635	0.9421	0.0358	694	0.4990	0.0233
518	0.1165	0.0142	577	0.7551	0.0354	636	0.9378	0.0357	695	0.4907	0.0231
519	0.1232	0.0148	578	0.7655	0.0355	637	0.9330	0.0357	696	0.4815	0.0227
520	0.1304	0.0154	579	0.7756	0.0355	638	0.9285	0.0356	697	0.4755	0.0225
521	0.1374	0.0160	580	0.7861	0.0355	639	0.9228	0.0354	698	0.4681	0.0223
522	0.1446	0.0165	581	0.7981	0.0357	640	0.9159	0.0353	699	0.4591	0.0220
523	0.1525	0.0172	582	0.8086	0.0357	641	0.9099	0.0351	700	0.4524	0.0217
524	0.1601	0.0177	583	0.8183	0.0357	642	0.9035	0.0350	701	0.4437	0.0214
525	0.1681	0.0183	584	0.8275	0.0358	643	0.8977	0.0349	702	0.4381	0.0212
526	0.1765	0.0189	585	0.8339	0.0357	644	0.8940	0.0348	703	0.4319	0.0210
527	0.1840	0.0194	586	0.8456	0.0358	645	0.8870	0.0347	704	0.4246	0.0208
528	0.1923	0.0200	587	0.8564	0.0359	646	0.8808	0.0345	705	0.4174	0.0205
529	0.2015	0.0206	588	0.8641	0.0359	647	0.8738	0.0344	706	0.4117	0.0203
530	0.2104	0.0211	589	0.8739	0.0360	648	0.8668	0.0342	707	0.4048	0.0201
531	0.2189	0.0216	590	0.8823	0.0360	649	0.8591	0.0340	708	0.3973	0.0198
532	0.2279	0.0222	591	0.8912	0.0361	650	0.8564	0.0340	709	0.3912	0.0196
533	0.2373	0.0227	592	0.8992	0.0361	651	0.8481	0.0338	710	0.3836	0.0193
534	0.2468	0.0232	593	0.9067	0.0362	652	0.8406	0.0336	711	0.3769	0.0191
535	0.2561	0.0237	594	0.9150	0.0363	653	0.8338	0.0334	712	0.3707	0.0188
536	0.2657	0.0242	595	0.9218	0.0363	654	0.8260	0.0332	713	0.3642	0.0186
537	0.2749	0.0246	596	0.9280	0.0363	655	0.8179	0.0330	714	0.3572	0.0183
538	0.2857	0.0251	597	0.9349	0.0364	656	0.8099	0.0328	715	0.3493	0.0180
539	0.2954	0.0255	598	0.9411	0.0364	657	0.8030	0.0326	716	0.3430	0.0178
540	0.3059	0.0260	599	0.9467	0.0364	658	0.7969	0.0325	717	0.3372	0.0176
541	0.3158	0.0264	600	0.9523	0.0365	659	0.7880	0.0322	718	0.3318	0.0174
542	0.3268	0.0269	601	0.9579	0.0365	660	0.7796	0.0320	719	0.3257	0.0172
543	0.3368	0.0272	602	0.9625	0.0366	661	0.7700	0.0317	720	0.3197	0.0170
544	0.3482	0.0277	603	0.9676	0.0366	662	0.7621	0.0315	721	0.3125	0.0167
545	0.3591	0.0280	604	0.9734	0.0367	663	0.7556	0.0313	722	0.3081	0.0165
546	0.3698	0.0284	605	0.9775	0.0367	664	0.7464	0.0311	723	0.3017	0.0163
547	0.3810	0.0288	606	0.9803	0.0367	665	0.7383	0.0308	724	0.2962	0.0161
548	0.3929	0.0292	607	0.9822	0.0367	666	0.7297	0.0306	725	0.2929	0.0160
549	0.4038	0.0295	608	0.9862	0.0368	667	0.7190	0.0302	726	0.2859	0.0157
550	0.4163	0.0299	609	0.9889	0.0368	668	0.7111	0.0300	727	0.2798	0.0155
551	0.4288	0.0302	610	0.9905	0.0368	669	0.7025	0.0298	728	0.2738	0.0152
552	0.4418	0.0306	611	0.9930	0.0369	670	0.6943	0.0295	729	0.2695	0.0151
553	0.4536	0.0309	612	0.9956	0.0369	671	0.6874	0.0294	730	0.2645	0.0149
554	0.4655	0.0312	613	0.9970	0.0369	672	0.6779	0.0291	731	0.2588	0.0147
555	0.4780	0.0315	614	0.9974	0.0369	673	0.6685	0.0288	732	0.2553	0.0145
556	0.4909	0.0318	615	1.0014	0.0371	674	0.6604	0.0285	733	0.2481	0.0143
557	0.5035	0.0321	616	1.0000	0.0370	675	0.6497	0.0282	734	0.2448	0.0141
558	0.5161	0.0323	617	0.9999	0.0370	676	0.6411	0.0279	735	0.2397	0.0139

Table 1 Continued

λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}	λ_{EM}	E	U_{95}
736	0.2338	0.0137	753	0.1702	0.0113	770	0.1220	0.0094	787	0.0876	0.0081
737	0.2288	0.0135	754	0.1678	0.0112	771	0.1194	0.0093	788	0.0862	0.0080
738	0.2251	0.0134	755	0.1649	0.0111	772	0.1186	0.0093	789	0.0850	0.0080
739	0.2222	0.0133	756	0.1607	0.0109	773	0.1164	0.0092	790	0.0838	0.0079
740	0.2168	0.0131	757	0.1574	0.0108	774	0.1131	0.0091	791	0.0814	0.0079
741	0.2123	0.0129	758	0.1536	0.0107	775	0.1105	0.0090	792	0.0790	0.0077
742	0.2089	0.0128	759	0.1509	0.0105	776	0.1083	0.0089	793	0.0767	0.0077
743	0.2046	0.0126	760	0.1483	0.0105	777	0.1067	0.0088	794	0.0750	0.0075
744	0.2010	0.0125	761	0.1452	0.0103	778	0.1039	0.0087	795	0.0753	0.0075
745	0.1973	0.0123	762	0.1434	0.0103	779	0.1018	0.0086	796	0.0743	0.0076
746	0.1941	0.0122	763	0.1411	0.0102	780	0.1011	0.0086	797	0.0713	0.0075
747	0.1909	0.0121	764	0.1371	0.0100	781	0.0986	0.0086	798	0.0698	0.0073
748	0.1860	0.0119	765	0.1350	0.0099	782	0.0964	0.0084	799	0.0691	0.0073
749	0.1815	0.0117	766	0.1324	0.0098	783	0.0936	0.0084	800	0.0675	0.0073
750	0.1789	0.0116	767	0.1301	0.0097	784	0.0922	0.0082			
751	0.1759	0.0115	768	0.1278	0.0097	785	0.0903	0.0082			
752	0.1730	0.0114	769	0.1252	0.0096	786	0.0886	0.0081			

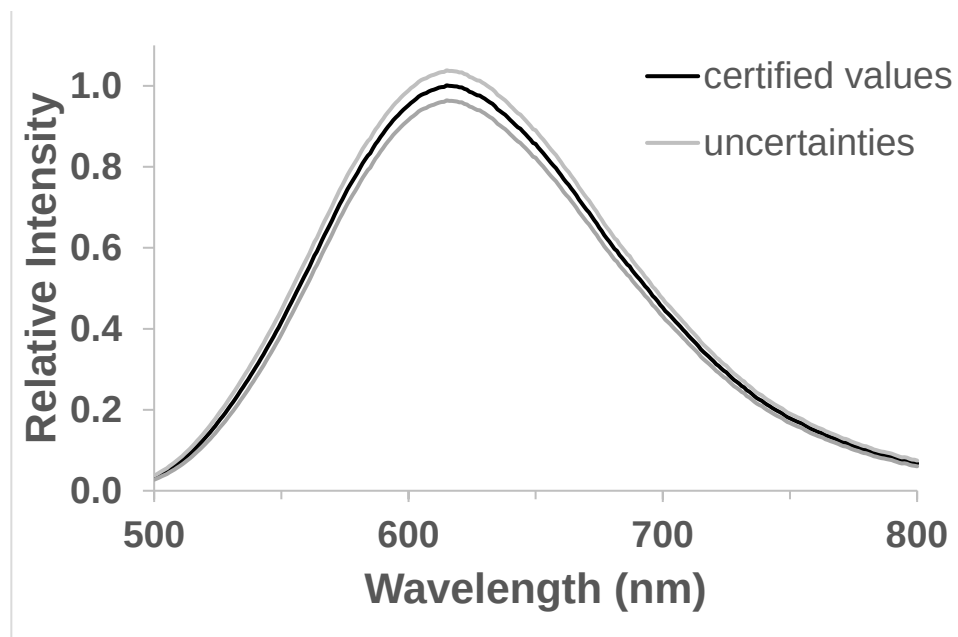


Figure 1. Spectrum for SRM 2940a Lot 3.

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 2940a 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 2940a

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

Physical Description: SRM 2940a is a manganese-doped (0.11 % MnO₂ 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 412.0 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 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 certified quantity E is the relative amount of fluorescence emitted from the sample, expressed in relative energy units. 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=2940a.

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 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 <https://www.nist.gov/srm>.