

Standard Reference Material[®] 916b

Bilirubin

CERTIFICATE OF ANALYSIS

Purpose: This Standard Reference Material (SRM) is intended for use in the calibration and standardization of measurement procedures for the determination of bilirubin in clinical samples, and for assignment of bilirubin quantity values to in-house control materials.

Description: This SRM comprises neat unconjugated bilirubin that is certified as a chemical substance of known purity. A unit of SRM 916b consists of 100 mg of bilirubin.

Certified Value: 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 measurand is the mass fraction of unconjugated bilirubin (expressed as percent) in the material [2].

The certified value was determined using a quantitative ¹H-nuclear magnetic resonance spectroscopy (q¹H-NMR) primary ratio measurement procedure [3,4] and is metrologically traceable to the International System of Units (SI) unit of mass, expressed as mass fraction percent, through use of the NIST PS1 Primary Standard for quantitative NMR (Benzoic Acid) [5].

Constituent	Mass Fraction ^(a) (%)
Bilirubin	94.7 ± 0.7

^(a) Values are expressed as $x \pm U_{95\%}(x)$, where x is the certified value and $U_{95\%}(x)$ is the expanded uncertainty of the certified value. The certified value and uncertainty ($U_{95\%}$) specify the range of values attributable to the measurand with a confidence level of approximately 95 % [6]. For guidance on using and propagating this uncertainty, see reference 7.

Period of Validity: The certified value delivered by **SRM 916b** is valid within the measurement uncertainty specified until **31 August 2026**, provided the SRM is handled and stored in accordance with instructions given in this certificate. The certifications are nullified if the SRM is stored or used improperly, damaged, contaminated, or otherwise modified.

Maintenance of Certified Values: NIST will monitor this SRM to the end of the period of validity. 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.

Safety: SRM 916b IS INTENDED FOR RESEARCH USE.

Storage: The SRM should be stored in its original container, tightly closed, at temperatures at or below $-20\text{ }^{\circ}\text{C}$ when not in use. Bilirubin is light-sensitive and should not be exposed to direct light. The SRM should always be protected from moisture and heat. The SRM should thermally equilibrate to ambient room temperature ($\leq 30\text{ }^{\circ}\text{C}$) before opening the container for use. The container should be tightly reclosed immediately after sampling bilirubin from the vial. The SRM should be kept at temperatures above $-20\text{ }^{\circ}\text{C}$ for no more than two (2) hours if intended to be used again at a later date.

Use: SRM 916b, stored as described above, should be used without preliminary drying. The minimum sample size is 5 mg.

Source: The SRM source material was obtained from a commercial supplier.

Analysis: Analyses for chemical identity, purity, and assessment of homogeneity were performed by NIST using twelve units of SRM 916b Bilirubin, sampled at regular intervals across the entire production lot. A $q^1\text{H-NMR}$ measurement procedure using an internal standard approach was implemented for the determination of bilirubin mass fraction. The uncertainty in the certified purity value reflects the 95 % coverage interval of the $q^1\text{H-NMR}$ measurement result, calculated using a Bayesian statistical procedure [8,9]. No trend in mass fraction of bilirubin was observed amongst the SRM production lot with respect to filling order and there is no significant heterogeneity at the 95 % level of confidence.

The SRM contains less than 0.8 % of volatile impurities, predominantly water and chloroform. A survey of elements attributable to impurities was conducted via wavelength-dispersive X-ray fluorescence spectroscopy and inductively coupled plasma mass spectrometry. The total mass fraction of these elements was determined to be approximately 0.6 %, primarily comprising chlorine (as chloroform), sodium, and potassium. This estimate is consistent with the determined chloroform content and ash residual measured via thermogravimetry.

Molar absorptivity values of SRM 916b Bilirubin in caffeine reagent and of the blue and red azopigment products were measured in accordance with methods described by [10] and [11] at Children's Wisconsin, Reference Standards Laboratory, Milwaukee, WI. The measured values of molar absorptivity, adjusted for bilirubin purity, are consistent with the non-certified Reference Values for Molar Absorptivities of SRM 916a Bilirubin and its azopigments at 200 mg/L [12,13]. This comparison verified the suitability of SRM 916b Bilirubin and its certified purity value for use with spectrophotometric measurement procedures for the determination of total bilirubin in clinical samples.

Details of the production of this SRM are described in NIST Special Publication 260-212, available at <https://doi.org/10.6028/NIST.SP.260-212> [13].

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If you use this SRM in published work, please reference:

Nelson MA, Brown Thomas J, Lang BE, Mulloor J, Sieber JR, Toman B, Yu LL, Lo S (2021) Certification of Standard Reference Material 916b Bilirubin. (National Institute of Standards and Technology, Gaithersburg, MD), NIST Special Publication (SP) 260-212. <https://doi.org/10.6028/NIST.SP.260-212>

Certain commercial equipment, instruments, or materials may be identified in this Certificate of Analysis 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.

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the Office of Reference Materials 100 Bureau Drive, Stop 2300, Gaithersburg, MD 20899-2300; telephone (301) 975-2200; e-mail srminfo@nist.gov; or the Internet at <https://www.nist.gov/srm>.

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