



Material Information Sheet

Reference Material

OMIC-1

Reference Material for SARS-CoV-2 Omicron BA.4/5 Spike Glycoprotein

OMIC-1 is a reference material (RM) designed for the development of SARS-CoV-2 BA.4/5 spike glycoprotein detection methods, as well as an antigen source for use in SARS-CoV-2 immunological assays. The reference values for OMIC-1 shown in Table 1 are based on combined results from two orthogonal methods from data generated at the National Research Council of Canada (NRC) [1]. A unit of OMIC-1 contains 0.2 mL of SARS-CoV-2 BA.4/5 spike glycoprotein in Dulbecco's phosphate-buffered saline (DPBS) with 10 mM HEPES sodium salt.

Table 1: Reference values and expanded uncertainties ($k = 2$) for OMIC-1

Compound	Molar concentration $\mu\text{mol/L}$	Mass fraction mg/g	Mass concentration $\text{mg/mL (21 }^\circ\text{C)}$
SARS-CoV-2 BA.4/5 spike protein (a,b) ¹	5.4 ± 0.5	0.75 ± 0.07	0.76 ± 0.07
SARS-CoV-2 BA.4/5 spike glycoprotein (a,b) ²	5.4 ± 0.5	0.97 ± 0.10	0.97 ± 0.10

¹SARS-CoV-2 BA.4/5 spike protein sequence only ($141\,324 \pm 1$ g/mol), the glycan molar mass is not included

²SARS-CoV-2 BA.4/5 spike glycoprotein total molar mass ($181\,000 \pm 7\,000$ g/mol), which includes the best estimate of the glycan molar mass

Refer to the sections below for additional explanations

Period of validity: until November 2032

Storage conditions: -80 °C

Table 2: Information values for OMIC-1

Compound	Relative amount %
trimer (c)	88
high molecular weight species (c)	8
low molecular weight species (c)	4

Intended use

Distributed in 0.2 mL units, this reference material is primarily intended for use in development, validation, and deployment of SARS-CoV-2 immunological tests.

Preparation of material

The protein was produced in mammalian (CHO) cells and purified at the NRC Human Health Therapeutics Research Centre [2, 3]. The solution was dispensed in 0.2 mL aliquots in sterile polypropylene vials with externally threaded screw tops. All sample processing was done in a sterile environment with pre-sterilized equipment.

Characterization of material

The explanatory list of letters next to each compound (Tables 1 and 2) refers to the instrumental method used for measurements:

- a) Amino acid analysis (AAA) by double isotope dilution liquid chromatography tandem mass spectrometry (LC-ID-MS/MS)
- b) Ultraviolet-visible spectrophotometry (UV-Vis)
- c) Size-exclusion liquid chromatography ultraviolet-visible spectrophotometry (LC-SEC-UV)

Mass concentration values were calculated using a density value of 1.005 ± 0.008 g/mL ($k = 2$) at 21 °C at the NRC on the RM formulation buffer. To account for the significant uncertainty in the mass of the attached glycans, the mass concentration of the glycoprotein was calculated using a total molar mass of $181\,000 \pm 7\,000$ g/mol.

Metrological traceability

Results presented in this certificate are traceable to the International System of Units (SI) through gravimetrically prepared standards of NRC CRMs APRO-1 (L-proline), ALEU-1 (L-leucine), and APHE-1 (L-phenylalanine), employed as calibration standards for LC-ID-MS/MS.

Homogeneity

The material was evaluated for homogeneity at the NRC using UV-Vis. Results from a representative number of ampules across the fill series were evaluated using the Bayesian analysis of variance (ANOVA) [4]. The between-unit variability was determined to be negligible.

Stability

The transportation stability of OMIC-1 was assessed using LC-SEC-UV at one-, seven-, and fourteen-day time points using an isochronous approach at +40, +20, +4, and -20 °C temperatures with reference to samples held at -80 °C. Significant changes in protein size heterogeneity were observed after seven days at +20 °C. It is advised to maintain the material in a frozen state until needed, however it can be safely kept at +4 °C for 24 hours within the scope of an experiment, such as in an autosampler. The material is shipped on dry ice, however the

OMIC-1 stability uncertainty includes an estimate of short-term instability at +40 °C to represent any significant shipping delay.

Freeze-thaw (F/T) stability was also tested via LC-SEC-UV. Reference samples were thawed as detailed below. F/T samples were analysed after undergoing an additional 1, 2, 3, 4, and 5 cycles with a freeze temperature of –80 °C. Small changes in size heterogeneity were observed after 4 F/T cycles, therefore limiting F/T cycles to 3 is recommended.

The long-term stability of OMIC-1 stored at –80 °C for thirty months was assessed using UV-Vis and compared to the initial assigned molar concentration. No significant differences in the measured concentration were observed over this period. Additionally, the LC-SEC-UV results of the isochronous study described above were supplemented with measurements from samples held at both –20 and –80 °C for ten weeks, as well as twelve and thirty months. A simple first-order degradation Arrhenius model was fitted to the data to make predictions for potential degradation of OMIC-1 after ten years at –80 °C. This long-term uncertainty component was included in the final stability uncertainty for OMIC-1.

Note that LC-SEC-UV reports only on the size heterogeneity of the material and the results from alternative structural or functional methods may differ.

Uncertainty

The expanded uncertainty (U) for all values is equal to $U = ku_c$, where u_c is the combined standard uncertainty calculated according to the Joint Committee for Guides in Metrology (JCGM) [5] and k is the coverage factor. A coverage factor of $k = 2$ was applied which corresponds to a level of confidence of approximately 95 %.

Included in the combined uncertainty estimates of the reference values in Table 1 are uncertainties in the batch characterization, uncertainties related to possible between-unit variation, and uncertainties related to stability.

Storage

The material shall be stored at –80 °C, however short-term (< 2 months) storage at –20 °C is acceptable. Extended storage at –20 °C may lead to protein aggregation.

Instructions for handling and use

Prior to opening, the vial should be thawed at room temperature until no residual ice crystals remain. The vial should then be gently inverted five times to ensure the solution is thoroughly mixed. To avoid foaming, vigorous mixing of the vial is discouraged. The vial should then be briefly spun in a centrifuge to remove any liquid that may be adhered to the cap. Once opened, the contents of the vial should be used immediately or aliquoted and re-frozen. It is recommended to not exceed three freeze-thaw cycles, as detailed above. Please note that the volume of the solution is not certified. Therefore, the entire contents of the vial should not be diluted to volume.

Health and safety information

Only qualified personnel should handle the material and appropriate disposal methods should be used. A Safety Data Sheet (SDS) is available at doi.org/10.4224/crm.2023.omic-1. For laboratory use only; not for human consumption, therapeutic, drug, household, or any other uses.

Period of validity

The reference values are valid until November 2032, provided the storage and instructions for handling and use specified in this certificate are followed.

Quality management system

The NRC is Canada's national metrology institute (NMI) and is a signatory of the International Committee for Weights and Measures Mutual Recognition Arrangement (CIPM MRA). The CIPM MRA was developed in a response to a growing need for an open, transparent, and comprehensive scheme to give users reliable quantitative information on the comparability of national metrology services and to provide the technical basis for wider agreements negotiated for international trade, commerce, and regulatory affairs. Our Quality Management System for measurement services and certified reference materials conforms to the requirements of ISO/IEC 17025 and ISO 17034.

Description of terms

Reference values are those for which not all uncertainty contributions may have been fully investigated or metrological traceability has not been fully established by the NRC.

Information values are those that may be of interest to users, but for which the NRC has not established sufficient information to provide an estimate of uncertainty, or that reflect a lack of agreement between different methods of analysis.

Supplemental information

Bibliographic information and any additional technical supplemental information such as the amino acid sequence of the OMIC-1 protein construct is available at doi.org/10.4224/crm.2023.omic-1.

References

1. Stocks BB, Thibeault M-P, L'Abbé D, et al. Characterization of biotinylated human ACE2 and SARS-CoV-2 Omicron BA.4/5 spike protein reference materials. *Anal Bioanal Chem*. 2024, 416: 4861–4872. <https://doi.org/10.1007/s00216-024-05413-7>
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3. Isho B, Abe K, Zuo M, et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Sci Immunol*. 2020, 5: eabe5511. <https://doi.org/10.1126/sciimmunol.abe5511>
4. van der Veen AMH. Bayesian analysis of homogeneity studies in the production of reference materials. *Accred Qual Assur* (2017), 22 (6): 307–19. <https://doi.org/10.1007/s00769-017-1292-6>
5. JCGM 100:2008. Evaluation of measurement data – Guide to the expression of uncertainty in measurement. Joint Committee for Guides in Metrology (JCGM); 2008. <https://doi.org/10.59161/JCGM100-2008E>

Authorship

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Approved by: _____

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This certificate is only valid if the corresponding material was obtained directly from the NRC or an authorized reseller. Users should ensure that the certificate they have is current. For updates, please refer to doi.org/10.4224/crm.2023.omic-1.

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