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Certificate of Analysis

Certified Reference Material

FLUNP-1

Calibration Solution Certified Reference Material for Influenza A (H5N1) Nucleoprotein

FLUNP-1 is a certified reference material (CRM) designed for use in influenza A nucleoprotein quantitative assays and as a positive control for point-of-care rapid antigen detection tests. The certified values for FLUNP-1 shown in Table 1 are based on data generated at the National Research Council of Canada (NRC). A unit of FLUNP-1 contains 0.2 mL recombinant influenza A (H5N1) nucleoprotein in 50 mM Tris buffer (pH 8.2), 150 mM sodium chloride, 50 mM L-arginine, and 50 mM L-glutamic acid.

Table 1: Certified values and expanded uncertainties (*k* = 2) for FLUNP-1

Compound	Molar concentration µmol/L	Mass concentration µg/mL (22 °C)
influenza A (H5N1) nucleoprotein (a)	3.8 ± 0.2	231 ± 13

Refer to the sections below for additional explanations

Period of validity: until February 2028
Storage conditions: –80 °C

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Table 2: Information values for FLUNP-1

Compound	Relative amount %
nucleoprotein oligomer (b)	84
nucleoprotein monomer (b)	16

Table 3: Information value for FLUNP-1

Compound	Mass concentration µg/mL (22 °C)
total host-cell proteins (c)	9

Intended use

Distributed in 0.2 mL units, this certified reference material is primarily intended for use in development and validation of influenza A nucleoprotein quantitative assays, and as a positive control for point-of-care rapid antigen detection tests.

Preparation of material

The protein was expressed in *E. coli* and purified from the cell lysate at the NRC Metrology Research Centre. The solution was dispensed in 0.2 mL aliquots into sterile polypropylene vials with externally threaded screw tops. All sample processing was performed in a sterile environment with pre-sterilized equipment.

Characterization of material

The explanatory list of letters next to each compound (Tables 1 to 3) refers to the instrumental method used for measurements:

a) Protein impurity-corrected amino acid analysis by isotope dilution liquid chromatography–tandem mass spectrometry (LC–ID–MS/MS)

b) Size-exclusion liquid chromatography–ultraviolet-visible spectrophotometry (LC–SEC–UV)

c) Liquid chromatography–tandem mass spectrometry (LC–MS/MS)

The molar and mass concentration values were calculated using the average molar mass of 60 531 ± 1 g/mol, determined from the amino acid sequence of the protein, and a density of 1.014 ± 0.002 g/mL (k = 2) at 22 °C determined at the NRC on the CRM formulation buffer.

Metrological traceability

Results presented in this certificate are traceable to the International System of Units (SI) through gravimetrically prepared standards of NRC CRMs APRO-2 (L-proline), ALEU-1 (L-leucine), and APHE-1 (L-phenylalanine) employed as calibration standards for LC–ID–MS/MS [1].

Homogeneity

The material was evaluated for homogeneity at the NRC using ultraviolet-visible spectrophotometry. Results from a representative number of units across the fill series were evaluated using the Bayesian analysis of variance (BANOVA) [2] and included as an uncertainty contribution to the certified values.

Stability

The transportation stability of FLUNP-1 was assessed using LC–SEC–UV at one-, seven-, and fourteen-day time points using an isochronous approach at temperatures of +40, +20, +4, and −20 °C with reference to samples held at –80 °C. Changes to size heterogeneity were observed after one day at +4 °C and significant decrease in protein signal was observed after one day at +40 °C. It is advised to maintain the material in a frozen state until needed. The material is shipped on dry ice; however, the FLUNP-1 stability uncertainty includes an estimate of transport instability at +20 °C for two days to represent any significant shipping delay.

The long-term stability of FLUNP-1 was evaluated using results of the isochronous study described above, supplemented with measurements from samples held at –20 and –80 °C for two and six months. A simple first-order degradation Arrhenius model was fitted to the data to make predictions for potential degradation of FLUNP-1 after two years at –20 °C. This long-term uncertainty component was included in the final stability uncertainty for FLUNP-1. This estimate is a conservative projection of possible instability, especially at –20 °C, as the long-term stability of the material under such conditions has not yet been rigorously assessed. Storage in a freezer at approximately –80 °C is therefore recommended.

Freeze-thaw (F/T) stability was also tested via LC–SEC–UV. Reference samples were thawed as detailed in the “Instructions for handling and use” section. F/T samples were analysed after undergoing an additional 1, 2, 3, 4, and 5 cycles with a freeze temperature of –80 °C. No significant changes in protein size heterogeneity were observed. However, a small decrease in total protein signal was observed with each additional F/T cycle suggesting protein loss due to aggregation or degradation. Therefore, F/T cycles should be minimized.

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) [3] and k is the coverage factor. A coverage factor of $k = 2$ was applied, which corresponds to a level of confidence of approximately 95 %.

All reasonable sources of uncertainty related to the certified values in Table 1 were considered. Included in the combined uncertainty estimate 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 (< 1 year) 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 refrozen. It is recommended to minimize freeze-thaw cycles, as detailed in the “Stability” section. It is important to note that the volume of the solution is not certified; only the concentration is 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.2026.flunp-1. For laboratory use only; not for human consumption, therapeutic, drug, household, or any other uses.

Period of validity

The certified values are valid until February 2028, 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

Certified values are those for which the NRC has the highest confidence and that all known and suspected sources of bias have been considered by the NRC and are reflected in the stated expanded uncertainties.

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 additional technical information, such as the nucleoprotein amino acid sequence, are available at doi.org/10.4224/crm.2026.flunp-1.

References

1. Thibeault M-P, Melanson JE, Stocks BB. Impurity-corrected ^{1}H-qNMR value assignment of amino acid certified reference materials for SI-traceable protein quantitation. Accred Qual Assur. 2026; in press. https://doi.org/10.1007/s00769-026-01714-9
2. van der Veen AMH. Bayesian analysis of homogeneity studies in the production of reference materials. Accred Qual Assur. 2017; 22 (6): 307–319. https://doi.org/10.1007/s00769-017-1292-6
3. 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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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.2026.flunp-1.

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