

National Institute of Advanced Industrial Science and Technology

## National Metrology Institute of Japan



## Reference Material Certificate

NMIJ CRM 6209-a  
No. +++

## Human Insulin Solution

This certified reference material (CRM) is produced in accordance with the NMIJ's management system and is in compliance with ISO 17034 and ISO/IEC 17025. It is an aqueous solution of human insulin. This CRM is primarily intended for use in the calibration and validation of analytical methods and instruments used for the determination of human insulin by instrumental analyses such as the amino acid analysis, the chromatography, and the spectrophotometry. It is also intended for use in controlling the precision of analytical methods and instruments. In addition, this CRM can be used in the validation of analytical instruments and evaluation of the accuracy of quantitative values in the analysis of human insulin by the immunoassay, after the commutability has been verified by the user.

**Certified Value**

The certified value, expressed as mass concentration of human insulin at 25 °C, is given in the table below. The uncertainty of the certified value is the half-width of the expanded uncertainty interval calculated using a coverage factor ( $k$ ) of 2, which gives a level of confidence of approximately 95 %.

	CAS No.	Certified value, Mass concentration (mg/L)	Expanded uncertainty, Mass concentration (mg/L)
Human insulin	11061-68-0	77.9	2.3

**Analysis**

The sample was hydrolyzed by hydrochloric acid to hydrolysate, which was evaluated by the amino acid analyses using the isotope-dilution mass spectrometry. To obtain the certified value, the results of the amino acid analyses were converted to the mass concentration by applying the density and molecular weight.

The hydrolysis and the amino acid analyses employed the following two different methods to quantify the hydrolyzed amino acids—aspartic acid, glutamic acid, valine, isoleucine, leucine, and phenylalanine.

(1) Liquid-phase hydrolysis, followed by the reversed-phase chromatography/tandem mass spectrometry utilizing the pre-column derivatization method:

The liquid-phase hydrolysis was performed by using hydrochloric acid at 150 °C for 48 h. The hydrolyzed amino acids were quantified by using *N*-butylnicotinic acid *N*-hydroxysuccinimide ester iodide as a derivatization reagent.

(2) Gas-phase hydrolysis, followed by the reversed-phase chromatography/tandem mass spectrometry utilizing the pre-column derivatization method:

The gas-phase hydrolysis was performed by using hydrochloric acid at 130 °C for 48 h. The hydrolyzed amino acids were modified with 1-bromobutane used as a derivatization reagent and quantified.

**Metrological Traceability**

The certified value of this CRM is traceable to the International System of Units (SI) via the amino acid analyses based on the isotope-dilution mass spectrometry which is the primary method, calibrated with amino acid CRMs: L-aspartic acid (NMIJ CRM 6027-a), L-glutamic acid (NMIJ CRM 6026-a), L-valine (NMIJ CRM 6015-a), L-isoleucine (NMIJ CRM 6013-a), L-leucine (NMIJ CRM 6012-a), and L-phenylalanine (NMIJ CRM 6014-a).

**Expiration of Certification**

The certificate is valid for one year from the date of shipment, provided that the material remains unopened and is stored in accordance with the instructions given in this certificate.

**Sample Form**

This CRM is in the form of a clear and colorless liquid. This CRM of ca. 1 mL in net volume is kept in a glass ampule, and the ampule is sealed in a polystyrene tube.

**Homogeneity**

The homogeneity of this CRM was verified by analyzing ten ampules taken from 189 ampules of the whole batch with the stratified random sampling method. Specifically, 0.1 g of this CRM was withdrawn from each of the ten ampules, and human insulin was measured with the reversed-phase chromatography. The homogeneity has been incorporated into the uncertainty of the certified value.

**Stability**

The stability was verified by the stability study at  $-20\text{ }^{\circ}\text{C}$ . The stability has been incorporated into the uncertainty of the certified value.

**Instructions for Storage**

This CRM should be stored at a temperature of around  $-20\text{ }^{\circ}\text{C}$ .

**Instructions for Use**

This CRM is for laboratory use only and it is not intended for *in vivo* use. The CRM should be thawed at room temperature for 2-3 hours before use and mixed thoroughly by gentle inversion of its ampule before use. This CRM should be used promptly once the ampule is opened. Care must be taken to prevent absorption onto labware when the CRM is diluted.

**Precautions for Handling**

Use a mask, gloves, and other personal protective equipment when handling this CRM. Refer to the safety data sheet (SDS) on this CRM before use.

**Preparation**

Commercial recombinant human insulin (Wako Pure Chemical Industries, Ltd.) was additionally purified by Peptide Institute, Inc. The solution was prepared by dissolving purified human insulin in phosphate buffer, and it was dispensed into glass ampoules in NMIJ.

**Technical Information**

- (1) The solution composition of this CRM is 20 mmol/L phosphate buffer (pH 7.4 at  $21\text{ }^{\circ}\text{C}$ ).
- (2) The density of this CRM is  $0.9995\text{ g/cm}^3$  at  $25\text{ }^{\circ}\text{C}$ .
- (3) The molecular weight of human insulin is 5807.6. The relative molecular mass of this CRM measured by the mass spectrometry was close to the molecular weight.
- (4) The concentration of this CRM without considering the molecular weight and density is  $(13.4 \pm 0.4)\text{ nmol/g}$ . [The number following  $\pm$  represents the expanded uncertainty with a coverage factor ( $k$ ) of 2.]

**NMIJ Analysts**

The technical manager for this CRM is TAKATSU A. and the production manager is SAKAGUCHI Y. The analysts are SAKAGUCHI Y., KINUMI T., MIZUNO R. and YOSHIOKA M.

**Information**

If substantive technical changes occur that affect the certification before the expiration of this certificate, NMIJ will notify the registered customer. Customer registration on the NMIJ Website (given below) will facilitate notification. Technical reports

regarding this CRM can be obtained from the contact details given below.

**Reproduction of Certificate**

In reproducing this certificate, it should be clearly indicated that the document is a copy.

April 1, 2020

ISHIMURA Kazuhiko

President

National Institute of Advanced Industrial Science and Technology

If you have any questions about this CRM, please contact:  
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