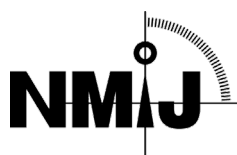


National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan



Reference Material Certificate

NMIJ CRM 6205-b
No. +++

Deoxyribonucleic Acid (DNA) Solutions for Quantitative Analysis (1 ng/μL, 600-bp)

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. This CRM consists of two kinds of deoxyribonucleic acid (DNA) solutions having different sequences. This CRM is principally intended to be used to control of the precision of DNA analytical instruments and methods such as DNA microarray (DNA chip), quantitative polymerase chain reaction (qPCR), and digital PCR.

Certified Values

The certified values for the mass concentration of total DNA (i.e., whole DNA materials in the sample solution regardless of sequence) of the two solutions (DNA600-G and DNA600-T) at 25 °C are given in the table below. The uncertainty of the certified value is the expanded uncertainty obtained by multiplying the combined standard uncertainty by a coverage factor (k) of 2, and it is the half-width of an interval estimated to have a level of confidence of approximately 95 %.

Sample name	Concentration as Total DNA	
	Certified value	Expanded uncertainty
	Mass concentration (ng/μL)	Mass concentration (ng/μL)
DNA600-G	0.88	0.07
DNA600-T	0.89	0.08

DNA600-G and DNA600-T were the solutions of DNA designed and prepared as shown in Figs. 1 and 2 of Technical Information.

Analysis

The certified value for each material was determined by converting the mass fraction of total DNA obtained by the following two analytical methods into the mass concentration using its density:

(1) Isotope dilution mass spectrometry (IDMS)

The mass fractions of dNMPs were obtained from liquid chromatograph-tandem mass spectrometry measurements via nucleobases produced by formic acid hydrolysis of DNA. Then, the mass fraction of DNA was calculated from the sum of the dNMP mass fractions, taking into account the dehydration at the phosphate ester bond.

(2) Inductively coupled plasma mass spectrometry (ICP-MS)

The mass fraction of phosphorus in each solution was quantified by ICP-MS after acid digestion. The mass concentration of the total DNA was calculated from the determined mass fraction of the total phosphorus content and number of phosphorus including target DNA.

Metrological Traceability

The certified values of this CRM were determined by IDMS and ICP-MS. IDMS was calibrated with the deoxynucleotide standard solutions which concentrations were evaluated by ¹H NMR in NMIJ. ¹H NMR used the internal standards calibrated with potassium hydrogen phthalate CRM (NMIJ CRM 3001-b). ICP-MS was calibrated with a phosphate ion standard solution of the Japan Calibration Service System (JCSS). The certified values, therefore, are traceable to the International System of Units (SI).

Indicative Values

Indicative values of this CRM are the amount of substance concentration at 25 °C as 600-bp DNA molecules having sequences shown in Figs. 1 and 2 of Technical Information. Those values were obtained by using the certified values and base contents in DNA molecules shown in Technical Information. The quoted uncertainty is the expanded uncertainty obtained by multiplying the combined standard uncertainty by a coverage factor (k) of 2.

Sample name	Concentration as 600-bp DNA	
	Indicative value Amount of substance concentration (fmol/μL)	Expanded uncertainty Amount of substance concentration (fmol/μL)
DNA600-G	2.37	0.17
DNA600-T	2.40	0.22

Expiration of Certification

This 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.

Description of the Material

This CRM is in the form of a clear and colorless liquid containing 600-bp synthesized double-stranded DNA dissolved in 10 mM Tris-buffer (pH8.5). Approximately 200 μL of each solution was bottled in a half transparent plastic vial, and a set of two solutions was kept in a decompression hermetically sealed nylon bag. This bag was then kept in an aluminum-laminated bag.

Instructions for Storage

This CRM should be kept in a freezer (−30 °C to −20 °C).

Instructions for Use

Prior to use, the frozen solution to be analyzed should be allowed to stand at room temperature (25 °C ± 5 °C) until it thaws (heating is strictly forbidden). After confirming that the cap of the vial is tightly closed, the vial is turned upside down gently several times for complete mixing. The thawed solution should be used immediately and is for single use only. The use of low-adsorption and DNase-free pipette tips and vials is recommended. This CRM is intended for *in vitro* laboratory use only.

Precautions for Handling

Refer to the safety data sheet (SDS) on this CRM before use.

Preparation

This CRM was designed, synthesized, purified, and bottled by Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST). The sequence is based on the artificial DNA sequence CRM 6203-aT, which was designed as random sequences that did not code for specific genes and the 300th base in the sequence was substituted for different bases. These DNAs were synthesized by polymerase chain reaction using NMIJ CRM 6205-a DNA600-G and DNA600-T as template, and purified by purification columns. Then, the purified DNA solutions were diluted by 10 mM Tris-HCl (pH 8.5). Approximately 200 μL of each solution was bottled in a half transparent plastic vial, and a set of two solutions was kept in a decompression hermetically sealed nylon bag. This bag was then kept in an aluminum-laminated bag.

Technical Information**(1) Sequence analysis**

This CRM consists of two kind of DNA solutions having different sequences. The 300th base in the sequence of DNA molecule in DNA600-G and DNA600-T solution, which was pointed out with dotted circle in Figs. 1 and 2, was substituted for different bases. The sequences of the prepared materials were analyzed by using automated DNA sequencer, and the sequences of both kinds of the materials were confirmed as the same sequence as designed one. The accession numbers of the database concerning the sequence (DDBJ/GenBank/EMBL) were AB610938 and AB610935, respectively. The molecular weights of DNA molecules in DNA600-G and DNA600-T solutions were 370773.3 and 370772.3, respectively.

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1  ATTCGAAGGG TGATTGGATC GGAGATAGGA TGGGTCAATC GTAGGGACAA TCGAAGCCAG
61 AATGCAAGGG TCAATGGTAC GCAGAATGGA TGGCACTTAG CTAGCCAGTT AGGATCCGAC
121 TATCCAAGCG TGTATCGTAC GGTGTATGCT TCGGAGTAAC GATCGCACTA AGCATGGCTC
181 AATCCTAGGC TGATAGGTTT GCACATAGCA TGCCACATAC GATCCGTGAT TGCTAGCGTG
241 ATTCGTACCG AGAACTCAGC CTTATGACT GCCCTTATGT CACCGCTTAT GTCTCCCGAG
301 ATCACACCCG TTATCTCAGC CCTAATCTCT GCGGTTTAGT CTGGCCTTAA TCCATGCCTC
361 ATAGCTACCC TCATACCATC GTCATACCT TCCGACATTG CATCCGTCAT TCCAACCCCTG
421 ATTCCTACGG TCTAACCTAG CCTCTATCCT ACCCAGTTAG GTTGCCTCTT AGCATCCCTG
481 TTACGTACGC TCTTACCATG CGTCTTACCT TGGCACTATC GATGGGAGTA TGGTAGCGAG
541 TATGGAACGG ACTAACGTAG GCAGTAAGCT AGGGTGTAAAG GTTGGGACTA AGGATGCCAG

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Molecular weight : 370773.3

Accession number: AB610938 (named as CRM 6203-a-G in the database)

Fig. 1 Sequence of DNA molecule in DNA600-G

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1  ATTCGAAGGG TGATTGGATC GGAGATAGGA TGGGTCAATC GTAGGGACAA TCGAAGCCAG
61 AATGCAAGGG TCAATGGTAC GCAGAATGGA TGGCACTTAG CTAGCCAGTT AGGATCCGAC
121 TATCCAAGCG TGTATCGTAC GGTGTATGCT TCGGAGTAAC GATCGCACTA AGCATGGCTC
181 AATCCTAGGC TGATAGGTTT GCACATAGCA TGCCACATAC GATCCGTGAT TGCTAGCGTG
241 ATTCGTACCG AGAACTCAGC CTTATGACT GCCCTTATGT CACCGCTTAT GTCTCCCGAG
301 ATCACACCCG TTATCTCAGC CCTAATCTCT GCGGTTTAGT CTGGCCTTAA TCCATGCCTC
361 ATAGCTACCC TCATACCATC GTCATACCT TCCGACATTG CATCCGTCAT TCCAACCCCTG
421 ATTCCTACGG TCTAACCTAG CCTCTATCCT ACCCAGTTAG GTTGCCTCTT AGCATCCCTG
481 TTACGTACGC TCTTACCATG CGTCTTACCT TGGCACTATC GATGGGAGTA TGGTAGCGAG
541 TATGGAACGG ACTAACGTAG GCAGTAAGCT AGGGTGTAAAG GTTGGGACTA AGGATGCCAG

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Molecular weight: 370772.3

Accession number: AB610935 (named as CRM 6203-a-T in the database)

Fig. 2 Sequence of DNA molecule in DNA600-T

(2) HPLC analysis

This CRM was analyzed by using size exclusion chromatography, and the single peak nearby 600-bp was obtained.

(3) Density measurement

The density of each solution at 25 °C was 0.9975 g/cm³, respectively.

NMIJ Analysts

The technical manager for this CRM is KATO M. and the production manager is SHIBAYAMA S. The analysts are SHIBAYAMA S., FUJII S., ZHU Y., YAMAZAKI T., NODAN., MATSUKURA S., SASAKI A., YOSHIOKA M. and TAKAGI T.

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.

December 23, 2021

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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