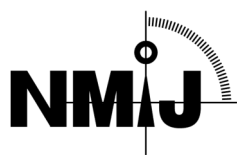


National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan



Reference Material Certificate

NMIJ CRM 6205-a
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 solutions of deoxyribonucleic acid (DNA) having different sequences. This CRM is principally intended to be used for the quality control or the validation of DNA analytical methods such as DNA microarrays (DNA chips) and quantitative polymerase chain reactions (qPCR).

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 half-width of the expanded uncertainty interval calculated using a coverage factor (k) of 2, which gives a level of confidence of approximately 95 %.

Sample name	Total DNA	
	Certified value Mass concentration (ng/μL)	Expanded uncertainty Mass concentration (ng/μL)
DNA600-G	1.38	0.12
DNA600-T	1.09	0.10

DNA600-G and DNA600-T are the solutions of DNA designed and prepared as shown in Fig. 1 and Fig. 2 in this certificate.

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)

Nucleobases, which were obtained from DNA by formic acid hydrolysis, were quantified by liquid chromatography tandem mass spectrometry (LC/MS/MS).

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

The phosphorus content 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.

Metrological Traceability

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

Indicative Values

Indicative values for the amount of substance concentrations for concentrations for the 600-bp DNA molecules at 25 °C are given in the table below. Those values were obtained by using the certified values and base contents in DNA molecules shown in the Technical Information. The uncertainty of the indicative 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 %.

Sample name	As 600-bp DNA	
	Indicative value	Expanded uncertainty
	Amount of substance concentration (fmol/ μ L)	Amount of substance concentration (fmol/ μ L)
DNA600-G	3.73	0.31
DNA600-T	2.97	0.27

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.

Sample Form

This CRM is in the form of a clear and colorless liquid at room temperature. This CRM of ca. 200 μ L in net volume is kept in a semi-transparent plastic vial, and a set of two solutions is sealed in a nylon bag hermetically by decompression. The bag is then sealed in an aluminum-laminated plastic bag.

Homogeneity

The homogeneity of this CRM was evaluated by measuring the DNA peak area by high performance liquid chromatography (HPLC), analyzing 10 vials randomly selected from 100 vials. The homogeneity is reflected in the uncertainty of the certified value.

Instructions for Storage

This CRM should be stored in a freezer at a temperature between $-30\text{ }^{\circ}\text{C}$ and $-20\text{ }^{\circ}\text{C}$.

Instructions for Use

Prior to use, the frozen solution to be analyzed should be allowed to warm to room temperature ($25\text{ }^{\circ}\text{C} \pm 5\text{ }^{\circ}\text{C}$) until it thaws (heating is strictly forbidden). After confirming that the cap of the vial is tightly closed, the vial is gently inverted several times to ensure 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 laboratory use only and not for *in vivo* use.

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 sequences were designed as random sequences that did not code for specific genes and the 300th base in the sequence was substituted for different bases. After these DNAs were inserted into a plasmid, the plasmid was duplicated in *E. coli*, extracted, purified, and fragmented with a restriction enzyme. The target DNAs, which were generated from the fragmented plasmid, were purified.

Technical Information

(1) Sequence analysis

The DNA sequences of DNA600-G and DNA600-T are shown in Figs. 1 and 2. The 300th base in the sequence, which is highlighted by a dotted circle in Figs. 1 and 2, was substituted for different bases (DNA600-G; guanine (G) and DNA600-T; thymine (T)). The sequences of the prepared materials were analyzed using an automated DNA sequencer, and the sequences of the isolated materials were confirmed to be the same as the designed sequences. The database accession numbers (DDBJ/GenBank/EMBL) of the CRM sequences are AB610938 and AB610935, respectively. The molecular weights of DNA600-G and DNA600-T are 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 TGATAGGTTC GCACATAGCA TGCCACATAC GATCCGTGAT TGCTAGCGTG
241 ATTCGTACCG AGAACTCAGC CTTATGACT GCCCTTATGT CACCGCTTAT GTCTCCCGAG
301 ATCACACCCG TTATCTCAGC CCTAATCTCT GCGGTTTAGT CTGGCCTTAA TCCATGCCTC
361 ATAGCTACCC TCATACCATC GCTCATACCT TCCGACATTG CATCCGTCAT TCCAACCCTG
421 ATTCCTACGG TCTAACCTAG CCTCTATCCT ACCCAGTTAG GTTGCCTCTT AGCATCCCTG
481 TTACGTACGC TCTTACCATG CGTCTTACCT TGGCACTATC GATGGGAGTA TGGTAGCGAG
541 TATGGAACGG ACTAACGTAG GCAGTAAGCT AGGGTGTAAG GTTGGGACTA AGGATGCCAG

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Fig. 1 Sequence of CRM 6205-a 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 TGATAGGTTC GCACATAGCA TGCCACATAC GATCCGTGAT TGCTAGCGTG
241 ATTCGTACCG AGAACTCAGC CTTATGACT GCCCTTATGT CACCGCTTAT GTCTCCCGAG
301 ATCACACCCG TTATCTCAGC CCTAATCTCT GCGGTTTAGT CTGGCCTTAA TCCATGCCTC
361 ATAGCTACCC TCATACCATC GCTCATACCT TCCGACATTG CATCCGTCAT TCCAACCCTG
421 ATTCCTACGG TCTAACCTAG CCTCTATCCT ACCCAGTTAG GTTGCCTCTT AGCATCCCTG
481 TTACGTACGC TCTTACCATG CGTCTTACCT TGGCACTATC GATGGGAGTA TGGTAGCGAG
541 TATGGAACGG ACTAACGTAG GCAGTAAGCT AGGGTGTAAG GTTGGGACTA AGGATGCCAG

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Fig. 2 Sequence of CRM 6205-a DNA600-T

(2) HPLC analysis

This CRM was analyzed by size exclusion chromatography column. A single peak nearby 600-bp was obtained.

(3) Density measurement

The densities of these solutions at 25 °C were 0.997 g/cm³.

NMIJ Analysts

For this CRM, the technical manager is TAKATSU A., the production manager is SHIBAYAMA S. and the analysts are SHIBAYAMA S., FUJII S., INAGAKI K., YAMAZAKI T., SEKIGUCHI Y., NODAN., MATSUKURA S., SASAKIA. and YOSHIOKAM.

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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Sample