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Shipping date : 20**.**.**

National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan

Reference Material Certificate

NMIJ CRM 6203-a
No. +++

National Institute of Advanced Industrial Science and Technology
AIST

Deoxyribonucleic Acid (DNA) Solutions for Quantitative Analysis

This certified reference material (CRM) was produced based on NMIJ's quality system in compliance with ISO GUIDE 34:2000 and ISO/IEC 17025:2005, and consists of the four kinds of deoxyribonucleic acid (DNA) solutions having different sequence. This CRM is principally intended to be used to assign the value of DNA calibrator for the evaluation and the control of the precision of the DNA analysis by using DNA microarray (DNA chip). It can also be used for controlling the precision and confirming the validity of analytical instruments and methods on DNA quantification such as a quantitative polymerase chain reaction (qPCR).

Certified Values

The certified values of four solutions (D001-600-A, -C, -G, and -T) for the mass concentration of total DNA (whole DNA materials in the sample solution regardless of base pair or sequence) at 20 °C are given in the following table. The expanded uncertainty was determined using coverage factor k =2, corresponding to an estimated confidence interval of approximately 95 %.

	Mass concentration of total DNA	
	Certified value (ng/μL)	Expanded uncertainty (ng/μL)
D001-600-A	12.4	0.8
D001-600-C	11.9	1.0
D001-600-G	12.4	1.1
D001-600-T	13.0	1.4

Analytical methods

The certified values of each material are based on the results by following analytical method:

(1) Isotope dilution - mass spectrometry (ID-MS)

Deoxyribonucleotides, which were enzymatically digested from DNA, were quantified by liquid chromatography mass spectrometry (LC/MS). The mass fraction of the total DNA was calculated based on the mass fraction of deoxyribonucleotides of DNA.

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

Phosphorus in the solution was quantified by ICP-MS after acid digestion. The mass fraction of the total DNA was calculated based on the mass fraction of phosphorus from DNA in the solution.

Mass concentration of the total DNA was calculated from obtained mass fraction of the total DNA and density of the solution.

Metrological Traceability

Each certified value is traceable to the International System of Units (SI) via deoxyribonucleotides analysis based on ID-MS as a primary method of measurement with standard solution of deoxyribonucleotides, purity of which were determined in NMIJ, and phosphorus analysis with phosphate ion standard solution of Japan calibration service system (JCSS).

Stability

The stability was confirmed during a period of an approximately a year. The stability will be monitored every six months.

Sample

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Expiration of Certification

This certificate is valid for 2 months after purchase under the specified storage condition below.

Sample Form

The form of this CRM is a clear and colorless liquid at room temperature. Approximately 50 μL of the each frozen solution was bottled in the half transparent plastic vial, and a set of 4 solutions was kept in an aluminum-laminated bag.

Homogeneity

The homogeneity of the CRM was determined by measuring DNA by HPLC, analyzing 6 vials selected from 80 vials. The homogeneity is reflected in the uncertainty of the certified value.

Storage

This CRM should be kept in a freezer (less than -20 °C) after purchase.

Instruction for Use

At prior to use, the frozen solution to be analyzed should be removed from the freezer and allowed to stand at room temperature (about 20 °C) until thawed (heating is strictly forbidden). After confirming the cap of the vial is tightly closed, the vial is turned upside down gently several times for complete mixing. Thawed solution should be used immediately and for single use only. Thawed solution should be sampled by using low-binding and DNase free pipet-tips and vials. NMIJ CRM 6203 is intended for *in vitro* laboratory use only.

Preparation Method

This CRM was designed, synthesized, purified, and bottled by bio-measurement research group, biomedical research institute, national institute of advanced industrial science and technology (AIST). The 300th base in the sequence was substituted for four different bases. These random sequences, that were not corded specific gene, were inserted into the plasmid, and the plasmid was duplicated in *E. coli*. The plasmid was extracted from *E. coli* and purified. The plasmid was fragmented with restriction enzyme, and the target sequence was purified.

Technical Information

(1) Sequence analysis

The basic sequence of this CRM is shown in Fig. 1 (e.g. CRM 6203-a-T). The 300th base in the sequence, which was pointed out with dotted circle in Fig. 1, was substituted for four different bases (D001-600-A; adenine (A), D001-600-C; cytosine (C), D001-600-G; guanine (G), and D001-600-T; thymine (T)). The sequences of the prepared materials were analyzed by using automated DNA sequencer, and the sequences of all kinds of the materials were confirmed as the same sequence as designed one.

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LOCUS       CRM 6203-a-T
ORIGIN
        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 AGAACTCACG CCTTATGACT GCCCTTATGT CACCGCTTAT GTCTCCCGAT
      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 design of CRM 6203-a-T (D001-600-T)

Sample

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(2) Gel electrophoresis

The CRM was analyzed by using polyacrylamide gel electrophoresis and microchip gel electrophoresis. The single band nearby 600 bp was obtained.

(3) Density analysis

The densities of these solutions at 20 °C are shown below. The numeric value after the symbol ± indicates expanded uncertainty ($k = 2$).

D001-600-A:0.9978 ± 0.0005 g/cm^3, D001-600-C:0.9983 ± 0.0005 g/cm^3, D001-600-G:0.9981 ± 0.0005 g/cm^3, D001-600-T:0.9983±0.0005 g/cm^3

(4) qPCR analysis (Evaluation of DNA contamination)

The evaluation of DNA contamination such as derived from plasmid and/or *E. coli* was performed by using qPCR method with specific primer set. The DNAs derived from plasmid and *E. coli* were less than 1 % and 0.03 %, respectively.

NMIJ Analysts

The technical manager for this CRM is A. Takatsu and production manager is S. Fujii. The analysts are S. Fujii, S. Shibayama, K. Inagaki, T. Narukawa, Y. Sekiguchi, M. Kawaharasaki, and M. Yoshioka.

Information

Customer registration on the NMIJ WEB site shown below will facilitate notification of above revision. Technical report about this CRM can be also obtained from the contact shown below.

Reproduction of Certificate

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

April 13, 2011

Tamotsu Nomakuchi
President
National Institute of Advanced Industrial Science and Technology

If you have any questions about this CRM, please contact
National Institute of Advanced Industrial Science and Technology,
National Metrology Institute of Japan,
Metrology Management Centre, Reference Materials Office,
1-1-1, Umezono, Tsukuba, Ibaraki 305-8563, Japan
Phone: +81-29-861-4059; Fax: +81-29-861-4009, http://www.nmij.jp/english/service/C/

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Sample