National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan

Reference Material Certificate

NMIJ CRM 6204-a
No. +++

National Institute of Advanced Industrial Science and Technology
AIST

Ribonucleic Acid (RNA) Solutions for Quantitative Analysis

This certified reference material (CRM) was produced in accordance with the NMIJ's management system, and in compliance with ISO GUIDE 34:2009 and ISO/IEC 17025:2005. This CRM consists of five kinds of ribonucleic acid (RNA) solutions having different lengths (533 or 1033 bases of single-strand RNA) and sequences. This CRM is principally intended to be used to assign the value of an RNA calibrator for the evaluation and control of the precision of RNA analytical methods such as DNA microarray (DNA chip), quantitative reverse-transcription PCR method, and next-generation DNA sequencer.

Certified Values

The certified values of five solutions (RNA500-A, -B, -C, RNA1000-A, and -B) for the mass concentration of total RNA (whole RNA materials in the sample solution regardless of sequence) 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 %. Purity (in mass fraction) of L-arginine is given as follows.

Sample name	Mass concentration of total RNA	
	Certified value (ng/μL)	Expanded uncertainty (ng/μL)
RNA500-A	30.6	3.1
RNA500-B	27.3	2.4
RNA500-C	32.4	3.2
RNA1000-A	58.3	4.9
RNA1000-B	59.5	5.3

Analysis

The certified values for each material were based on the results obtained by the following analytical methods:

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

Ribonucleotides, which were obtained from RNA by using enzymatic digestion, were quantified by liquid chromatography mass spectrometry (LC/MS).

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

Phosphorus in the solution was quantified by ICP-MS after acid digestion.

The mass concentration of the total RNA was calculated from the obtained mass fraction of the total RNA and density of the solution.

Metrological Traceability

Each certified value was traceable to the International System of Units (SI) via ribonucleotides analysis based on ID-MS as the primary method of measurement and phosphorus analysis using the phosphate ion standard solution of the Japan Calibration Service System (JCSS). The standard solution of ribonucleotides, the purity of which was determined by impurity determination and phosphorus analysis using ICP-optical emission spectrometry, was employed.

Indicative Values

The amount of substance concentration of RNA molecules at 25 °C is given in the table below as an indicative value, which

was calculated using the certified value and base content in RNA molecules shown in 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	Amount of substance concentration of RNA	
	Indicative value (pmol/μL)	Expanded uncertainty (pmol/μL)
RNA500-A	0.178	0.019
RNA500-B	0.159	0.016
RNA500-C	0.189	0.020
RNA1000-A	0.175	0.015
RNA1000-B	0.179	0.018

Expiration of Certification

This certificate is valid for 3 months from the date of shipment, provided that the material remains unopened and 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. Approximately 300 μL of each solution was bottled in a half transparent plastic vial, and a set of five solutions was kept in a decompression hermetically sealed nylon bag. This bag was then kept in an aluminum-laminated bag.

Homogeneity

The homogeneity of the CRM was evaluated by measuring RNA by spectrophotometric analysis, analyzing 10 vials randomly selected from about 110 vials. The uncertainty of homogeneity was reflected in the uncertainty of the certified value.

Stability

The stability of the CRM was confirmed by NMIJ and the uncertainty of stability was reflected in the uncertainty of the certified value. The stability will be monitored.

Instruction for Storage

This CRM should be kept in a freezer (temperature lower than –20 °C) after delivery.

Instructions for Use

Prior to use, the frozen solution to be analyzed should be allowed to stand at room temperature (about 20 °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 thawed solution should be sampled using low-adsorption and RNase-free pipette tips and vials. NMIJ CRM 6204 is intended for *in vitro* laboratory use only.

Precaution for Handling

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

Preparation Method

This CRM was designed, synthesized, purified, and bottled by Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST). RNAs in five solutions had different lengths (533 or 1033 bases of single-strand RNA) and sequences. These random sequences, which were not coding-specific genes, were inserted into a plasmid and the plasmid was duplicated in *E. coli*. The plasmid was then extracted from *E. coli* and purified. Thereafter, the plasmid was fragmented with a restriction enzyme, and the target RNA sequence was synthesized enzymatically and purified.

Information

(1) Sequence analysis and molecular weight

The sequence, molecular weight, and accession number of the database concerning the sequence (DDBJ/GenBank/EMBL) of this CRM is shown in Fig. 1. The sequences of the complementary DNA (cDNA) synthesized using the prepared materials were analyzed using an automated DNA sequencer, and the sequences of all kinds of materials were confirmed to be the same as the designed one.

```
BASE COUNT      165 a    127 c    121 g    120 u
ORIGIN
        1 GGGCUCGACU AGUUAAUACG GUACAGGAUA ACCGAUCGGC UUGCAACAUA ACGGCGUUAA
       61 GAAUGCGGGA GUGCAGUUUC CGAUUCUCAC AUCAAUCGCC AAUAAGGCCU UGUCGCAAUA
      121 UAGACUCAAC GGUUCUAGUA GCUGAUCGGU AUUACGUGAC GCAACCGAUU AGACAUGCAC
      181 AAUUCCUUGG UCGCUAUACU ACGGAAAUCG UCAGGUACUA UAACCCGUCG CAGGCCUAAU
      241 ACGUGUCGUC ACAUCGCCAA CCUAUCGUCA GUCGGAAAGA CGUUGCUGUC UACCAUCGAA
      301 ACUAUUUACC GCUCCGAGAU UCACGAGUAC GAACUCACGA GGAAGUUGCC CUAUGUAAGG
      361 UAUCACUCCA GGUACUGCGC CGAUAGUACC AGGUGAUCAA ACGGUUGCAA GAAGGCCACG
      421 ACGUAUCGGG CUCUUUAGAC GUACGCUCGA GAUUAAACGC GCACUGAUUC ACUUUAGCCC
      481 GGAAUGUCUC GGUGCGAUGU AGAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAA
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Molecular weight: 171 603.8

Accession number: AB610939 (registered as 500-1)

Fig. 1(a). RNA500-A

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BASE COUNT      163 a    130 c    130 g    110 u
ORIGIN
        1 GGGAGACUAA AUCUCGGCGU CGGUUCAUAC GCGCGAUCGU UUGCUGUCAG GGCAUACUCG
       61 AAUCCGGACU CCGACAAUUA UAGGCCAUCC UGAAUAGCCG AUCAUGCGAG UCACGAUAAG
      121 GCAGGCUCUG CGAUAUCCCG AUAUACUGGA GAAGCUGAAU CCCACCUAGA GCGAACUGUC
      181 AGAGGAUCGA CCUCAGGCUC GCUAUCAUCA UAACGGCGGA CGACCUGUGU CACAUUCCGA
      241 ACGCUACGUG ACGAUAUUAU CUGUCGAAAG GCAUAGAACG CCGGUCAAUA UCCUGCGGCA
      301 UUCUCUUUAU CACCGGCUAU AACUACUAGG UUCCGCAGAU AUAGACUGCG CACGGAACAU
      361 GUAGAUAGAU CGAGUAGGGU AGCGAUUUAA CGACUCGACU UACAGACAGA GACGUAGAAC
      421 GUCAGACGAG UGGUAUGCCC ACCAGAGGCG AUACAGGCUG UACCUGCGUA GCACUAGAGU
      481 CGUGCGUCAU GCGGACCCUA UCUAAAAAAA AAAAAAAAAA AAAAAAAAAA AAA
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Molecular weight: 171 906.1

Accession number: AB610940 (registered as 500-2)

Fig. 1(b). RNA500-B

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BASE COUNT      171 a    126 c    116 g    120 u
ORIGIN
        1 GGGACUAAAC GCACUGAAUA CCGUACUACA ACAGACGAAG UUGUAAAUAG CCGUGGUAAU
       61 UAUGAACGAA UAUGGCCAUG UGUCCGCUAA UCCGCGGUAC UAGCCAGUUA GCAACUGCAC
      121 CAAUCGCUCA CGUCAGUGGU UCUAUGCAAU AUGCUCCAGU ACCCUGUAAG UUCGCAAUCA
      181 AUAGACGCGC CUUACUCCUC UCAAGAAGGG UAUCUGCAUG AGCCGACACA UCAAGACCCA
      241 AUGGACGUUU GAGCGAGUGG CUUGGAGAGU AUUAACGCAC UAACUCUUCG AAGGCUUACU
      301 UCGGCAAAUC CGCGAGCUCC ACUAUUAACA UGCCAAUACG ACAGGAUCAA UUCUGCGACU
      361 GCACGACCGA AUUAUGCACC UACUUUGUGA GGCACGAGAU UCGUCUUGCA GCUAUUUAAA
      421 GGGUUCCAGC UUAUGGAUAG GCGACUCUUC AGUGCGUAAU AAAGCAACGC CCAAUCGGCA
      481 UGUUACCGGA UAGUACGGGC GAUAAAAAAA AAAAAAAAAA AAAAAAAAAA AAA
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Molecular weight: 171 547.8

Accession number: AB610942 (registered as 500-4)

Fig. 1(c). RNA500-C

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BASE COUNT      283 a    258 c    257 g    235 u
ORIGIN
        1 GGGCGAUUCG AAGAGGUACG AGUGGACGCG UAAGCGAAUG ACCUAGACCU CGGCGUUAAU
       61 UAGGACCCUC UAAUCGCAAA CUCGACUCUC GUCCCAAUCC AAUGGAUGUC CAGUGCUCGG
      121 UAGCAUGAUC GUAUGAUGCG UAUCGCUGCG AGUAGAGGCC GACAAGUAGA CCGGUGCGAA
      181 UUUGGAGGUA CUUAGCCUCA UAUGAGAGCG CCUUGAAAUC ACCCAGUGCC GAUCGUAGCG
      241 GAAGAUUACU AGACUCCGCA GGGAAAUCCC ACCUGUAACG ACGGAAGAGC GUCACGAUAG
      301 CCUCUAACUA UCCGGUUCGC GACUAUCCGC UUAUGUGCCU CCACCUAAUG UGAGAGUUCA
      361 CCGAGGCAAA UGAUCUGUCA ACCGGUGUGA UCAGGACAUA CGCUUAAUGC CGUAGAAGCC
      421 CGUAAGCUCU CCGCCCUUUA AGAGGUUGUA GACGGCAGUU CUAAGGUCGU CGGGUCUAUG
      481 CCUUGCGACC UAAUAAUACG ACCGUGUGCU UAUGCGGACU GUCCUCUAAU GAAUAUCGCU
      541 UGUCCUAAGC UGGCGGUACU AGUGCUUAGG AUCGCACACC UCACCACAGU GCGCAUUUAA
      601 CCCUGUAGAU AACAUGGUAG ACACCGGUAA AUCGCGUUCG AAUUUCGCCC AAUCGAAGGC
      661 CCACAUCACU ACGUCGCCUG UAUUCUGAAC CUUGCGCUGC ACGUAGCAUA UAGAGCGUAC
      721 AUUCAAUCUA CCAGUUGCCU CCGACUGAAG UCGGCUAGCG UAUGACAUAG CGAGCUCUUA
      781 GUUCGGUGAC UACUUCUAGC ACUCCCAAUU CAAGCUCUGC GUUAUCAGGG UCGGAAGGUU
      841 AGGUUCGAAU UUCGACAGGC UAACAGAGCG AUAAGUGAUG AAUCCGCUCC GGGAGCAUCU
      901 AGACAAUAAC CGCGGUUAAG AGAAGGGCGA CAUAAGCGCG GGUGUCAACG UUCAAACCAG
      961 UUGUAGCCAU CGCGAUUACC CGUUGGGAAU CUGAGGCGAC CUAAAAAAAA AAAAAAAAAA
     1021 AAAAAAAAAA AAA
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Molecular weight: 332 585.9
Accession number: AB610946 (registered as 1000-3)

Fig. 1(d). RNA1000-A

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BASE COUNT      267 a    262 c    245 g    259 u
ORIGIN
        1 GGGAUUCCUA GGACUGUACU CUCGGUGCGU UGACCAUACG UAAGGCGAUC CUUUGAGUGG
       61 AUCCCAUUAC UACGCGUCAC ACCUGCUUAC CCUCCCAAUA GUUGGUUCAG UAGCUCUCAG
      121 CGGUUCUGGC AGAGUUCGGA UGAGUUUCUG CCUAUCAGUU CAUAGGUGCC CACGCAUUGG
      181 GUCCACUCCU CGCCAGAAUU UGCGCAUUGC ACCAUUACUA CAGGCGGCUU UGGUUGUACG
      241 UCUAACGUUC GCACCAACAG GAGUCUCAGC UGAUCAUAGG CCCGGACCCU CAAUGUUCGA
      301 UGCGAUUCGU AAGAGGGUGU UCGUGUAAGG CCCAAUACGU UGUCAUGCCG GCUUAGAAAC
      361 CCAGUCGGAC GCGUCUCUAA CACUCGGAUG UGCAGGUAAU AGCCUUUACC AGCGCUUCUG
      421 UACGACCAUA CUUAGAGCUC GAGAUGCCGA CAUGAAAGGA UUCCGGAGUA CUGACCUGAA
      481 UACACGUUCA UAGCGUAAAU CGGCCGAGAU UCAACUUUAC GGCACGGAUA CAGCUCCUCU
      541 ACCUAUUUCC GUCGAAGUCU CUCACGAUAG UCGCGUACAU UUAGUGGGCG GUACACACAG
      601 CACGUCAACG CCAUCGCACU CUGAGUUCCC ACUCCACGGU ACGUUCACAG CACGUUGCCU
      661 UAAUAAGCUA CUUCGGUUCC GAGCAGUCAA CCUACUGUUU CCGGGUUAGC GCUCUGAUCA
      721 GCACCCGUUU ACUGACACGA ACCGCUAUCG AAUACUGAGU AGGUCGUGUG CCAAUAACUU
      781 UGGUUGCAGC UAAGCUAAUC GGACGGCGAC UUUAGCAAGU AACUCAGCCG UAUUGUUACG
      841 CUGACCGUAA ACGACGUGAG CGAUUGUCGU AGGUUAGCCA UAACAUAAAG GUUUCCCGAA
      901 CGGUAGCAUA GUUAGGCCUG UGUCCAGUCA GGUAAUACGA GAGAGUAAUU AACGCGAUCU
      961 AAUGAGAAGC CGUGCAUGUC GAUCCUUGUU ACGGGUGUGA AAUAAAAAAA AAAAAAAAAA
     1021 AAAAAAAAAA AAA
//
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Molecular weight: 331 744.9
Accession number: AB610947 (registered as 1000-4)

Fig. 1(e). RNA1000-B

(2) Gel electrophoresis

The single bands near 533 or 1033 bases were obtained using polyacrylamide gel electrophoresis and microchip gel electrophoresis.

(3) Density measurement

The density of these solutions at 25 °C was 0.9971 g/cm^3.

NMIJ Analysts

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

Technical Information

Customer registration on the NMIJ Website (given below) will facilitate notification of any revision of the information given above. 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, 2015

Ryoji Chubachi
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,
Center for Quality Management of Metrology, Reference Materials Office,
1-1-1 Umezono, Tsukuba, Ibaraki 305-8563, Japan
Phone: +81-29-861-4059; Fax: +81-29-861-4009; https://www.nmij.jp/english/service/C/

Revision history
April 1, 2015: "Metrology Management Center" was renamed to "Center for Quality Management of Metrology."