National Institute of Advanced Industrial Science and Technology

National Metrology Institute of Japan

Reference Material Certificate

NMIJ CRM 6204-b
No. +++

Ribonucleic Acid (RNA) Solutions for Quantitative Analysis

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 five solutions of ribonucleic acid (RNA) having different lengths (533 or 1033 bases of single-strand RNA) and sequences. This CRM is primarily intended to be used in the evaluation, and control of the precision, of RNA analytical instruments and methods such as DNA microarrays (DNA chips), quantitative reverse-transcription PCR, and next-generation DNA sequencers. It can also be used to assign the value of an RNA sample for evaluation of DNA chips.

Certified Values

The certified values for the mass concentrations of total RNA (i.e., whole RNA materials in the sample solution regardless of sequence) of the five solutions (RNA500-A, -B, -C, RNA1000-A, and -B) at 25 °C are given in the table below. The uncertainty of the certified value is the expanded uncertainty 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 RNA	
	Certified value Mass concentration (ng/μL)	Expanded uncertainty Mass concentration (ng/μL)
RNA500-A	33.4	2.6
RNA500-B	32.3	2.6
RNA500-C	32.1	3.1
RNA1000-A	68.2	5.8
RNA1000-B	64.1	5.5

RNA500-A, -B, -C, RNA1000-A, and -B are the solutions of RNA designed and prepared as shown in Fig. 1 in this certificate.

Analysis

The certified values for each material were determined by converting the mass fractions of the total RNA obtained using the following analytical methods into the mass concentration using its density:

(1) Isotope dilution mass spectrometry (IDMS)

Ribonucleotides, which were obtained from RNA using enzymatic digestion, were quantified by liquid chromatography mass spectrometry (LC/MS). The mass fraction of RNA was calculated from the sum of the ribonucleotides mass fractions considering dehydration at phosphodiester bonds in RNA molecule.

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

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

Metrological Traceability

The certified values were determined by IDMS with the ribonucleotide standard solutions which were evaluated by ^{1}H NMR using internal standards calibrated with potassium hydrogen phthalate CRM (NMIJ CRM 3001-b), by ICP-MS with a phosphate ion standard solution from the Japan Calibration Service System (JCSS), and by density measurement with a density standard

liquid (water) from JCSS. The certified values, therefore, are traceable to the International System of Units (SI).

Indicative Values

The indicative values of this CRM are the amount of substance concentrations for the RNA molecules shown in Fig. 1, in this certificate, at 25 °C are given in the table below. These values were obtained by using the certified values and the base compositions in the RNA molecules shown in Technical Information.

The quoted uncertainty is the expanded uncertainty 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 a targeted RNA molecule	
	Indicative value Amount of substance concentration (pmol/μL)	Expanded uncertainty Amount of substance concentration (pmol/μL)
RNA500-A	0.195	0.014
RNA500-B	0.188	0.014
RNA500-C	0.187	0.017
RNA1000-A	0.205	0.015
RNA1000-B	0.193	0.014

Expiration of Certification

This certificate is valid for 6 months from the date of shipment, provided that this CRM 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 533 or 1033 bases synthesized single-stranded RNA dissolved in sterilized water at room temperature. This CRM of ca. 300 μL in net volume is kept in a semi-transparent plastic vial (for low-adsorption of nucleic acids), and a set of five solutions is sealed in a nylon bag hermetically by decompression. The bag is then sealed in an aluminum-laminated plastic bag.

Instructions for Storage

This CRM should be stored in a freezer at a temperature between –30 °C and –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 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 RNase-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 RNAs in the five solutions have different lengths (533 or 1033 bases of single-strand RNA) and sequences. These random sequences, which do not code for specific genes, were inserted into a plasmid, which 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. Then, the purified RNA solutions were diluted by sterilized water. 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.

Technical Information

(1) Sequence analysis and molecular weight

The sequences, molecular, and weights of RNAs for this CRM are shown in Fig. 1. The database accession numbers (DDBJ/GenBank/EMBL) of the template DNA used in the enzymatic synthesis of this CRM are also 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 were confirmed to be the same as the designed one.

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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 6204-a-500-1)

Fig. 1(a). Sequence of RNA molecule in 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 CRM6204-a-500-2)

Fig. 1(b). Sequence of RNA molecule in 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
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      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 6204-a-500-4)

Fig. 1(c). Sequence of RNA molecule in RNA500-C

```
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 6204-a-1000-3)

Fig. 1(d). Sequence of RNA molecule in 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
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 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 6204-a-1000-4)

Fig. 1(e). Sequence of RNA molecule in RNA1000-B

(2) Gel electrophoresis

The CRM material gave single bands near 533 or 1033 base using microchip gel electrophoresis.

(3) Density measurement

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

NMIJ Analysts

The technical manager is for this CRM is TAKATSU A., the production manager is FUJII S., and the analysts are FUJII S., SHIBAYAMA S., INAGAKI K., YAMAZAKI T., SEKIGUCHI Y., NODA N., MATSUKURA S., SASAKI A. 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

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