TÜBİTAK
ULUSAL METROLOJİ ENSTİTÜSÜ

Reference Material Data Sheet

Name of the Material : SARS-CoV-2 Synthetic RNA Fragment

Reference Material Code : UME RM 2019

Issue Date : 24.09.2020

Last Revision Date : 29.04.2021 *(Revision history can be found on the last page)*

Validity Period : 4 months from the sales date

Assigned Values :

Gene Region	Assigned Value[1] Cq	Standard Deviation[2] Cq
RdRp-IP2 (France)	24.73	0.35
RdRp-IP4 (France)	21.79	0.13
RdRp (Germany)	20.92	0.06
E (Germany)	19.64	0.26
N1 (USA CDC)	21.06	0.12
N2 (USA CDC)	22.70	0.29
Orf1ab (China CDC)	23.27	0.57
N (China CDC)	24.17	0.26
Orf1 (Hong Kong)	23.38	0.38
N (Hong Kong)	23.26	0.40
RNAse P (USA CDC)	22.42	0.29

[1] The assigned value is the average of the Cq values obtained by three replicate RT-qPCR measurements on three different days ($n = 9$) of the 1000-fold diluted solution of the material.

[2] The standard deviation of the assigned value is the standard deviation of the Cq values ($n = 9$) obtained in RT-qPCR measurements.

Sales Date

Dr. Mustafa ÇETİNTAŞ
Director

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Assigned Values :

Gene Region	Assigned Value[1] copy/µL	Standard Deviation[2] copy/µL
RdRp-IP2 (France)	8.4E+07	5.4E+06
RdRp-IP4 (France)	7.9E+07	4.3E+06
RdRp (Germany)	9.1E+07	1.1E+07
E (Germany)	6.1E+07	1.3E+06
N1 (USA CDC)	8.7E+07	8.5E+06
N2 (USA CDC)	7.2E+07	5.1E+06
Orf1ab (China CDC)	8.3E+07	2.2E+06
N (China CDC)	7.7E+07	6.3E+06
Orf1 (Hong Kong)	8.2E+07	4.4E+06
N (Hong Kong)	7.6E+07	5.7E+06
RNAse P (USA CDC)	8.5E+07	9.5E+06

[1] The assigned value is the average of the RNA concentration (copy/µL) obtained by three replicate RT-ddPCR measurements on two different days ($n = 6$) of 100000 fold diluted solution of the material.

[2] The standard deviation of the assigned value is the standard deviation of the RNA concentration (copy/µL) value ($n = 6$) obtained in RT-ddPCR measurements.

Description

SARS-CoV-2 Synthetic RNA Fragment (UME RM 2019) contains gene fragments of the SARS-CoV-2 virus used in the Reverse Transcription Real Time Polymerase Chain Reaction (RT-qPCR) and Reverse Transcription Digital Polymerase Chain Reaction (RT-dPCR) measurement protocols, which were released by World Health Organization (Appendix 1). The material consists of 1079 bases long single-stranded synthetic RNA containing 10 different SARS-CoV-2 gene fragments and human RNase P gene fragment (Appendix 2). The material was obtained by cloning the construct into the plasmid, linearizing the plasmid with a restriction enzyme and following *in vitro* transcription. The sequence of the material was verified by Sanger Sequencing method after reverse transcription and registered in the GenBank with accession number MW001217.

The material was filled in a volume of 100 µL in liquid form in 1 mM sodium citrate buffer (pH: 6.5) to which 2.5 ng/µL of total human control RNA (Thermo Fisher 4307281) was added. The reference material UME RM 2019 is sold frozen. The material contains approximately 10^8 copies/µL of the SARS-CoV-2 synthetic RNA fragment according to Qubit RNA fluorescence measurements.

Since the relative standard deviation is found to be 0.4% in the homogeneity test between units, it has been shown that the material has sufficient homogeneity suitable for the intended use.

For simulating transport conditions, the stability of the material was tested at -20 °C for 16 days, and no sign of degradation was observed.

For testing storage conditions, the material was kept at -20 °C for 4 months, and it was found stable.

As long as the material is on sale, stability will be checked, and customers will be informed in case of any instability doubt.

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Intended Use

The material is suitable for use as a positive quality control material in the measurement of SARS-CoV-2 virus, the agent of COVID-19 disease, by RT-qPCR and RT-dPCR methods including the reverse transcription step.

UME RM 2019 is not a certified reference material.

Instructions for Use

This material can be safely dispatched at -20 °C and below temperatures, provided that the transport time does not exceed two weeks.

Immediately after thawing the material on ice, it can be serially diluted 1000 times or at any dilution factor with nuclease (RNase and DNase) free pure water, and aliquoted into new tubes. The aliquots must be stored at -70 °C or lower temperature. All dilutions must be performed on ice.

The diluted frozen material should be thawed on ice for each experiment and kept on ice until added to the PCR tube. Material remaining in the tube should not be reused.

It should be used in a volume of at least 5 µL of the diluted reference material for each RT-qPCR or RT-dPCR.

1000 tubes with a volume of 100 µL can be obtained by 1000-fold dilution of the material. When using 5 µL for each RT-qPCR or RT-ddPCR, 20000 RT-qPCRs or RT-ddPCR can be performed with this reference material.

More information regarding the dilution of the reference material is shared with the customers during the shipment.

Storage Conditions

The material should be stored at -20 °C or lower temperatures.

It is recommended that the material is stored at -70 °C or lower after dilution.

TÜBİTAK UME cannot be held responsible for changes that might happen to the material at customer's premises due to noncompliance with the instructions for use, and the storage conditions described in the reference material data sheet.

Safety Information

UME RM 2019 contains small gene fragments of the SARS-CoV-2 virus and was produced *in vitro*. Since the material does not contain any virus alive, it is considered as non-infectious.

Usual laboratory precautions apply. It is strongly recommended that the material must be handled and disposed according to the safety guidelines where applicable.

Please read the Safety Data Sheet before use.

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Participants

Information about the laboratory participated in the sample preparation and measurement is presented in the following table.

Laboratory	Address
TÜBİTAK UME	TÜBİTAK Gebze Yerleşkesi, Barış Mah. Dr. Zeki Acar Cad. No.1 41470 Gebze - Kocaeli, Turkey

Techniques Used for the Determination of the Assigned Values

RT-qPCR was performed by using SensiFAST™ No-ROX One-Step Kit (Meridian Bioscience BIO-76001) with Roche LightCycler® 480 Real Time PCR instrument. One step RT-qPCR conditions are as follows: Reverse transcription 45 °C for 10 min (RdRp-Germany: 30 min); initial enzyme activation 95 °C for 5 min (RdRp-Germany: 15 min); denaturation and amplification 95 °C for 15 s and 58 °C for 45 s, respectively; and total of 50 denaturation and amplification cycles. The primer and probe sequences and the related information are given in Appendix 1.

The assigned Cq values determined for the gene regions contained in the material were obtained as a result of three-day and three-replicate measurements ($n = 9$) by RT-qPCR of the material prepared by diluting 1000 times as specified in instructions for use part. Standard deviation was calculated using the Cq values ($n = 9$) obtained for each gene and is given with the assigned values.

The assigned concentration of the material (copy/µL) was obtained as a result of measurements of different gene regions using the Reverse Transcription Droplet Digital Polymerase Chain Reaction (RT-ddPCR) method. RT-ddPCR measurements were performed using the QX200 BioRad PCR instrument and the One-Step RT-ddPCR Advanced Kit for Probes (BioRad, 1864021). RT-ddPCR conditions were as follows: Reverse transcription 50 °C for 60 min; enzyme activation 95 °C for 10 min; denaturation 95 °C for 30 s; amplification 58 °C for 1 min (E-Germany and N-China: 61 °C for 1 min); enzyme inactivation is at 98 °C for 10 min and 40 cycles in total. Double quenched ZEN-IBFQ probes were utilized in all RT-ddPCR measurements (Appendix 1). The concentration of the material was calculated after diluting by 100000 times by gravimetric serial dilutions and measuring them in two different days in three replicates ($n = 6$). Standard deviations were calculated using the RNA concentration (copy/µL) values determined for each gene contained in the material.

The assigned values are not certified values; they only provide information about the content of the reference material.

More detailed information about the reference material production and value assignment is given in the article [6].

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Revision History

Date	Explanation
24.09.2020	First publication.
11.11.2020	Change of assigned values with corrected reverse primer and new probe dye for RdRp (Germany) gene fragment. Reverse primer sequence correction and change of probe dye for RdRp (Germany) gene fragment analysis in Appendix 1. Addition of instrument model, kit information and RT-qPCR conditions. Addition of article correction: "Letter to the editor: SARS-CoV-2 detection by real-time RT-PCR" to Reference [2] for RdRp (Germany) gene fragment analysis.
29.04.2021	Extension of validity period to 4 months from the sales date. Addition of assigned RNA concentration (copy/µL) values after measurements with RT-ddPCR method. Update of assigned values with additional set of measurements. Addition of Qubit RNA fluorescence measurement method and information about the long-term stability test. Addition of details of RT-ddPCR experiment protocols to the techniques section Addition of a note about dilution on instructions for use section Addition of the article describing more details about the reference material.

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Appendix 1. Target gene regions, primer and probe sequence information used in SARS-CoV-2 synthetic RNA fragment production. Double quenched ZEN-IBFQ probes were utilized in all RT-ddPCR measurements.

Gene Region	Primer and Probe Sequences	Modification	References
RdRp-IP2 (France)	F-ATGAGCTTAGTCCTGTTG R-CTCCCTTTGTTGTGTTGT P-AGATGTCTTGTGCTGCCGGTA	HEX-BHQ-1	[1]
RdRp-IP4 (France)	F-GGTAACTGGTATGATTTCG R-CTGGTCAAGGTTAATATAGG P-TCATACAAACCACGCCAGG	FAM-BHQ-1	[1]
RdRp (Germany)	F-GTGARATGGTCATGTGTGGCGG R-CARATGTTAAARACACTATTAGCATA P-CAGGTGGAACCTCATCAGGAGATGC	FAM-ZEN-IBFQ	[2]*
E (Germany)	F-ACAGGTACGTTAATAGTTAATAGCGT R-ATATTGCAGCAGTACGCACACA P-ACACTAGCCATCCTTACTGCGCTTCG	FAM-ZEN-IBFQ	[2]
N1 (USA CDC)	F-GACCCCAAAATCAGCGAAAT R-TCTGGTTACTGCCAGTTGAATCTG P-ACCCCGCATTACGTTTGGTGGACC	FAM-ZEN-IBFQ	[3]
N2 (USA CDC)	F-TTACAAACATTGGCCGCAAA R-GCGCGACATTCCGAAGAA P-ACAATTTGCCCCCAGCGCTTCAG	FAM-ZEN-IBFQ	[3]
Orf1ab (China CDC)	F-CCCTGTGGGTTTTACACTTAA R-ACGATTGTGCATCAGCTGA P-CCGTCTGCGGTATGTGGAAAGGTTATGG	FAM-BHQ-1	[4]
N (China CDC)	F-GGGGAACTTCTCCTGCTAGAAT R-CAGACATTTTGCTCTCAAGCTG P-TTGCTGCTGCTTGACAGATT	FAM-BHQ-1	[4]
Orf1 (Hong Kong)	F-TGGGGYTTTACRGGTAACCT R-AACRCGCTTAACAAAGCACTC P-TAGTTGTGATGCWATCATGACTAG	FAM-BHQ-1	[5]
N (Hong Kong)	F-TAATCAGACAAGGAACTGATTA R-CGAAGGTGTGACTTCCATG P-GCAAATTGTGCAATTTGCGG	FAM-BHQ-1	[5]
RNAse P (USA CDC)	F-AGATTTGGACCTGCGAGCG R-GAGCGGCTGTCTCCACAAGT P-TTCTGACCTGAAGGCTCTGCGCG	HEX-BHQ-1	[3]

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Appendix 2. SARS-CoV-2 Synthetic RNA Fragment RNA Sequence

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GGGAGAAUGAGCUUAGUCCUGUUGCACUACGACAGAUGUCUUGUGCUGCCGGUACUACACAAACUGCUUG
CACUGAUGACAAUGCGUUAGCUUACUACAACACAACAAAGGGAGGUGACCCUGUGGGUUUUACACUUAAA
AACACAGUCUGUACCGUCUGCGGUAUGUGGAAAGGUUAUGGCUGUAGUUGUGAUCAACUCCGCGAACCCA
UGCUUCAGUCAGCUGAUGCACAAUCGUUUAUGGUAACUGGUAUGAUUUCGGUGAUUUCAUACAAACCACG
CCAGGUAGUGGAGUUCCUGUUGUAGAUUCUUAUUAUUCAUUGUUAAUGCCUAUAUUAACCUUGACCAGGG
GAGUGAAAUGGUCAUGUGUGGCGGUUCACUAUAUGUUAAACCAGGUGGAACCUCAUCAGGAGAUGCCACA
ACUGCUUAUGCUAAUAGUGUUUUUAACAUUUGUCAAUGGGGUUUUACAGGUAACCUACAAAGCAACCAUG
AUCUGUAUUGUCAAGUCCAUGGUAAUGCACAUGUAGCUAGUUGUGAUGCAAUCAUGACUAGGUGUCUAGC
UGUCCACGAGUGCUUUGUUAAGCGUGUUGAAGACAGGUACGUUAAUAGUUAAUAGCGUACUUCUUUUUCU
UGCUUUCGUGGUAUUCUUGCUAGUUACACUAGCCAUCCUUACUGCGCUUCGAUUGUGUGCGUACUGCUGC
AAUAUUGUGGACCCCAAAAUCAGCGAAAUGCACCCCGCAUUACGUUUGGUGGACCCUCAGAUUCAACUGG
CAGUAACCAGAAUUAGGGGAACUUCUCCUGCUAGAAUGGCUGGCAAUGGCGGUGAUGCUGCUCUUGCUUU
GCUGCUGCUUGACAGAUUGAACCAGCUUGAGAGCAAAAUGUCUGGUACUAAUCAGACAAGGAACUGAUUA
CAAACAUUGGCCGCAAAUUGCACAAUUUGCCCCCAGCGCUUCAGCGUUCUUCGGAAUGUCGCGCAUUGGC
AUGGAAGUCACACCUUCGCUGCAGCCGGGAGAUUUGGACCUGCGAGCGGGUUCUGACCUGAAGGCUCUGC
GCGGACUUGUGGAGACAGCCGCUCGGAUC
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References

[1] “Protocol: Real-time RT-PCR assays for the detection of SARS-CoV-2”, Pasteur Institute, Paris, France: https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf

[2] Corman V. M. et al., “Detection of 2019 novel coronavirus (2019-nCov) by real-time RT-PCR”, Euro Surveill. 2020, 25(3), 1-8, doi: 10.2807/1560-7917.ES.2020.25.3.2000045.
*Article Correction: "Letter to the editor: SARS-CoV-2 detection by real-time RT-PCR" in volume 25, 2000880, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7268274

[3] “2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes”, Centers for Disease Control and Prevention (CDC), Atlanta, GA, ABD, 2020.06.24:
https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html

[4] “Real-Time Fluorescence-based Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) assay of the 2019-nCoV Nucleic Acid”, Chinese Center for Disease Control and Prevention:
doi: 10.46234/ccdcw2020.085. 2020.03.13

[5] Leo Poon, Daniel Chu and Malik Peiris, “Protocol: Detection of 2019 novel coronavirus (2019-nCoV) in suspected human cases by RT-PCR”, School of Public Health, The University of Hong Kong, Hong Kong:
https://www.who.int/docs/default-source/coronaviruse/whoinhouseassays.pdf?sfvrsn=de3a76aa_2

[6] Akyurek, S., Demirci, S.N.S., Bayrak, Z. et al. The production and characterization of SARS-CoV-2 RNA reference material. Anal Bioanal Chem (2021). https://doi.org/10.1007/s00216-021-03284-w

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