

Certificate of Analysis

SARS-CoV-2 (N+E) RNA Quant Standard:

Part No.	Name	Size
AM205A	SARS-CoV-2 (N + E) RNA, 4×10^6 copies/ μ l	100 μ l



Instructions for use of this product can be found in the *GoTaq® Enviro Wastewater SARS-CoV-2 Systems Technical Manual, #TM661*, available online at: www.promega.com/protocols/

Description: SARS-CoV-2 (N+E) RNA Quant Standard (Cat.# AM2050), is an RNA fragment encoding the nucleocapsid (N) gene and the envelope (E) gene of SARS-CoV-2. The SARS-CoV-2 (N+E) RNA Quant Standard serves as a stable quantitation standard and is supplied at 4×10^6 copies/ μ l for generating a standard curve in an RT-qPCR amplification.

Expiration Date: See the product label for the expiration date.

Storage Conditions: Store at -30°C to -10°C .

Usage Note: For maximum product activity, do not exceed 5 freeze-thaw cycles.

Quality Control Assay

This lot passes the following quality control specifications:

Functional Assay: Replicate amplifications of SARS-CoV-2 (N+E) RNA in amounts from 2×10^5 to 20 copies per reaction must yield a standard curve with a slope of -3.3 ± 0.3 and $r^2 \geq 0.990$.

Part# 9PIAM205

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Signed by:

R. Wheeler, Quality Assurance

1. Preparing Standard RNA Dilutions for SARS-CoV-2 RT-qPCR Quantitation

To avoid contamination of samples with external sources of PCR templates, perform all steps with aerosol-resistant pipette tips. We recommend using low DNA binding tubes. We do not recommend storing diluted RNA. Always prepare fresh serial dilutions.

Materials to Be Supplied by the User

- nuclease-free, low-retention, qPCR-compatible reaction tubes or plates
- 0.5ml low-binding tubes (e.g., Eppendorf Cat.# 022431005)
- pipettes and sterile, aerosol-resistant tips
- Nuclease-Free Water (Cat.# P1193)
- RT-qPCR amplification reagents (e.g., GoTaq® Enviro RT-qPCR System, Cat.# AM2010, AM2011)
- qPCR thermal cycler

1. Dilute the SARS-CoV-2 (N+E) RNA Quant Standard, 4×10^6 copies/ μ l, 100-fold by mixing 2 μ l into 198 μ l of Nuclease-Free Water to obtain a concentration of 4×10^4 copies/ μ l.
2. Perform subsequent serial tenfold dilutions in low-binding 0.5ml tubes. For example, combine 5 μ l of RNA + 45 μ l of Nuclease-Free Water to obtain the standard curve dilutions as shown in Table 1 (4×10^4 –4 copies/ μ l).
3. Assemble RT-qPCR reactions as specified by manufacturer's instructions
4. Perform RT-qPCR thermal cycling as shown in Table 2. The PCR cycling parameters and instrument settings shown are provided as guidelines and can be modified as necessary for optimal results.

Table 1. Standard Curve Dilutions for SARS-CoV-2 (N+E) RNA.

SARS-CoV-2 (N + E) Copies/Well		Copies/Well
RNA (copies/ μ l)	(5 μ l in 20 μ l)	(4 μ l in 10 μ l)
4×10^4	2×10^5	1.6×10^5
4×10^3	2×10^4	1.6×10^4
4×10^2	2×10^3	1.6×10^3
40	2×10^2	1.6×10^2
4	20	16

Table 2. Example qPCR Cycling Conditions using GoTaq® Enviro Wastewater SARS-CoV-2 Systems (Cat.# AM2100).

Step	Temperature (°C)	Time	Number of Cycles
reverse transcription	45	15 minutes	1
RT inactivation/ GoTaq® activation	95	2 minutes	1
denaturation	95	15 seconds	40
annealing/extension	62	60 seconds	