## Certificate of Analysis

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

Part# 9PIAM205

Printed 11/21

| Part No. | Name | Size |
|---|---|---|
| AM205A | SARS-CoV-2 (N + E) RNA, $4 \times 10^6$ copies/µl | 100µl |

**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 $4 \times 10^6$ copies/µl 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.

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## 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 $2 \times 10^5$ to 20 copies per reaction must yield a standard curve with a slope of $-3.3 \pm 0.3$ and $r^2 \geq 0.990$.

Promega


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Signed by:

R. Wheeler, Quality Assurance


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# Usage Information

## 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, $4 \times 10^6$ copies/µl, 100-fold by mixing 2µl into 198µl of Nuclease-Free Water to obtain a concentration of $4 \times 10^4$ copies/µl.
2. Perform subsequent serial tenfold dilutions in low-binding 0.5ml tubes. For example, combine 5µl of RNA + 45µl of Nuclease-Free Water to obtain the standard curve dilutions as shown in Table 1 ($4 \times 10^4$–4 copies/µl).
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) RNA (copies/µl) | Copies/Well (5µl in 20µl) | Copies/Well (4µl in 10µl) |
|---|---|---|
| $4 \times 10^4$ | $2 \times 10^5$ | $1.6 \times 10^5$ |
| $4 \times 10^3$ | $2 \times 10^4$ | $1.6 \times 10^4$ |
| $4 \times 10^2$ | $2 \times 10^3$ | $1.6 \times 10^3$ |
| 40 | $2 \times 10^2$ | $1.6 \times 10^2$ |
| 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 | |

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