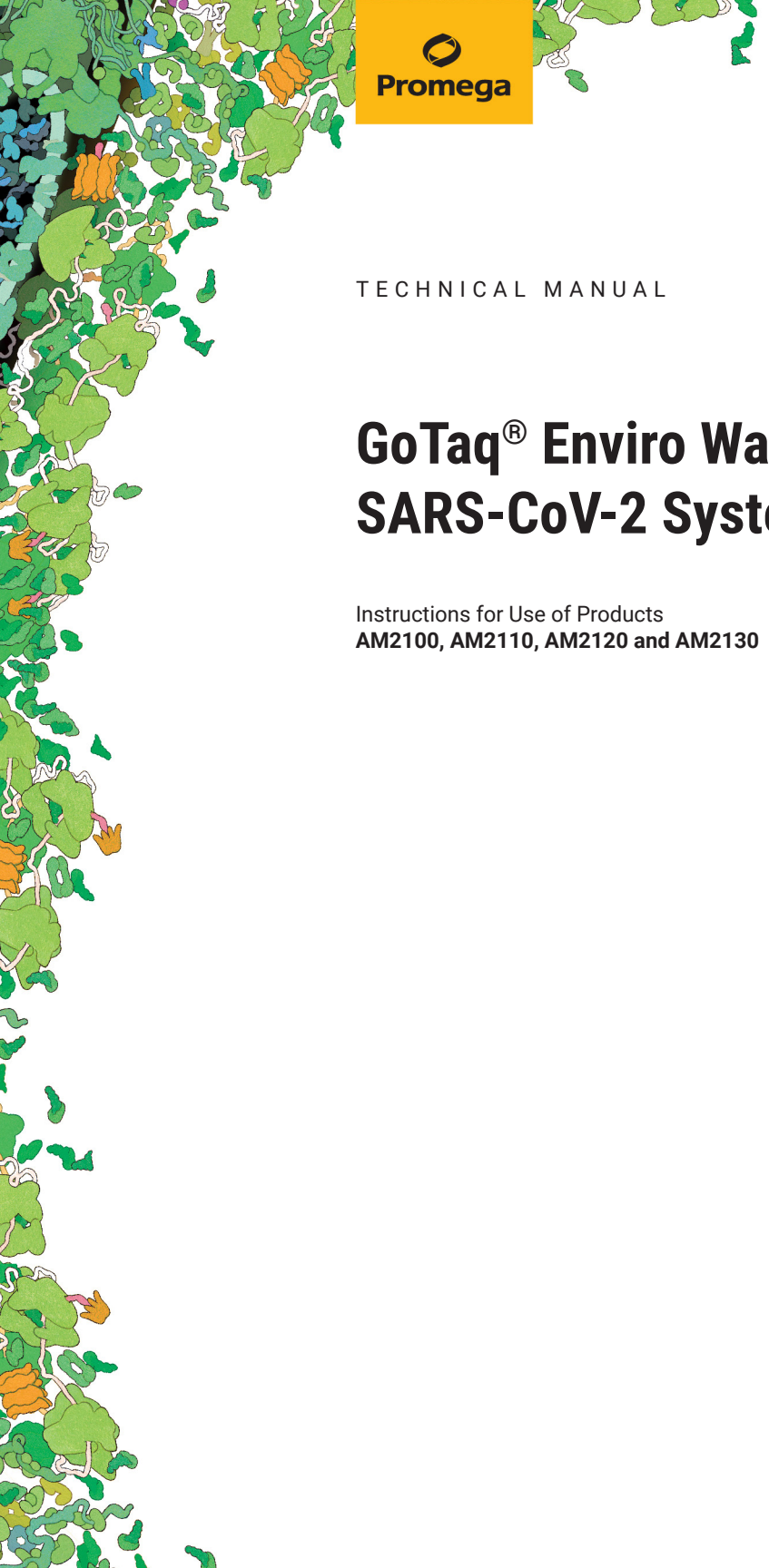

TECHNICAL MANUAL

GoTaq® Enviro Wastewater SARS-CoV-2 Systems

Instructions for Use of Products
AM2100, AM2110, AM2120 and AM2130

Revised 4/24
TM661

GoTaq® Enviro Wastewater SARS-CoV-2 Systems

All technical literature is available at: www.promega.com/protocols/
Visit the website to verify that you are using the most current version of this Technical Manual.
Email Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

Early in the COVID-19 pandemic, scientific studies demonstrated that the genetic material of SARS-CoV-2—an enveloped RNA virus—could frequently be detected in the feces of infected individuals (1–3) and thereafter was also detected in wastewater (4). This finding mobilized the global water sector to investigate if wastewater-based epidemiology (WBE) using the genetic signal of SARS-CoV-2 could be used to track the spread of COVID-19 in communities. In the past, WBE was critical in identifying the community prevalence of poliovirus in support of the World Health Organization's (WHO) program to eradicate poliomyelitis and was also used to investigate opioid use in communities (5). Detection of SARS-CoV-2 in wastewater has the potential to provide an integrated, community-level indication of the presence of COVID-19.

The GoTaq® Enviro Wastewater SARS-CoV-2 Systems for wastewater samples are four-dye, three-target hydrolysis probe-based reverse transcription (RT)-qPCR kits that amplify SARS-CoV-2 genetic signatures. The systems include primer/probe sets prescribed by the U.S. Centers for Disease Control and Prevention (CDC) that target the N1 or N2 regions of the SARS-CoV-2 nucleocapsid (N) gene, or a primer/probe set targeting the envelope (E) gene (6). The systems are designed for a one-step RT-qPCR. As an internal process control and to allow for normalization, the systems include primers to detect Pepper Mild Mottle Virus (PMMoV), an RNA virus commonly found in wastewater (7), and an internal amplification control (IAC). The GoTaq® Enviro Master Mix, provided in this kit, uses proprietary enzymes and formulations that tolerate reverse transcriptase and PCR inhibitors such as humic acids, that can be present in nucleic acid samples purified from wastewater.

Product Components (Section 2) and Related Products (Section 8.C) include:

Target Genes: Three different primer/probe sets are supplied as 20X Primer/Probe/IAC mixtures for detecting either the N1 or the N2 fragment of the nucleocapsid (N) gene, or the envelope protein (E) gene of SARS-CoV-2. These amplicons are detected using Fluorescein- (FAM™-) labeled probes.

Controls: Each 20X Primer/Probe/IAC Mix contains two controls:

- **Process Control**: Detection of the Pepper Mild Mottle Virus (PMMoV), an abundant RNA virus commonly found in wastewater. The primers and Quasar® 670 dye-labeled probe amplify and detect a 68bp region of the PMMoV genome and are detected in the Cy®5 channel.
- **Internal Amplification Control (IAC):** The 20X Primer/Probe/IAC Mix contain primers, probe and template for an internal amplification control (IAC). The primers and the CAL Fluor® 560 dye-labeled probe amplify and detect a 285bp product from an RNA template that is included in every amplification reaction. IAC performance is used to detect RT-qPCR inhibitors (*Taq* polymerase and reverse transcriptase inhibitors) in the sample and are detected in the HEX™/JOE™/VIC® dye channel.

Passive Reference Dye: The 20X Primer/Probe/IAC Mix includes a reference dye (CXR, carboxy-X-rhodamine) that has similar spectral properties to ROX™ dye. The final concentration of the Passive Reference dye in RT-qPCR is 30nM.

DNA Polymerase and Reverse Transcriptase: GoTaq® Enviro qPCR Master Mix contains thermostable DNA polymerase and the GoScript™ Enzyme Mix contains reverse transcriptase. These mixes are designed to tolerate a diverse range of DNA polymerase and reverse transcriptase inhibitors, including those found in wastewater.

RNA Quantitation Standards: GoTaq® Enviro Wastewater SARS-CoV-2 Systems contain two in vitro transcribed RNA fragments: SARS-CoV-2 (N+E) RNA, 4 × 10^6 copies/µl, and PMMoV RNA, 4 × 10^6 copies/µl. These RNA fragments serve as stable quantitation standards that can be used to generate a standard curve.

Nuclease-Free Water: Can be used as a negative no-template control (NTC), for diluting the quantitation standards and for adjusting the setup volume for RT-qPCR reaction mixes.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
GoTaq® Enviro Wastewater SARS-CoV-2 System, N1/N2/E	**200 reactions**	**AM2100**

Not For Medical Diagnostic Use. The system contains sufficient reagents for 200 reactions at 20µl. Includes:

- 1 × 100µl N1 & PMMoV Primer/Probe/IAC Mix, 20X
- 1 × 100µl N2 & PMMoV Primer/Probe/IAC Mix, 20X
- 1 × 100µl E & PMMoV Primer/Probe/IAC Mix, 20X
- 2 × 1,000µl GoTaq® Enviro Master Mix, 2X
- 1 × 100µl GoScript™ Enzyme Mix
- 2 × 1.25ml Nuclease-Free Water
- 1 × 100µl SARS-CoV-2 (N+E) RNA, 4 × 10^6 copies/µl
- 1 × 100µl PMMoV RNA, 4 × 10^6 copies/µl

PRODUCT	SIZE	CAT. #
GoTaq® Enviro Wastewater SARS-CoV-2 System, N1	**200 reactions**	**AM2110**

Not For Medical Diagnostic Use. The system contains sufficient reagents for 200 reactions at 20µl. Includes:

- 2 × 100µl N1 & PMMoV Primer/Probe/IAC Mix, 20X
- 2 × 1,000µl GoTaq® Enviro Master Mix, 2X
- 1 × 100µl GoScript™ Enzyme Mix
- 2 × 1.25ml Nuclease-Free Water
- 1 × 100µl SARS-CoV-2 (N+E) RNA, 4 × 10^6 copies/µl
- 1 × 100µl PMMoV RNA, 4 × 10^6 copies/µl

PRODUCT	SIZE	CAT. #
GoTaq® Enviro Wastewater SARS-CoV-2 System, N2	**200 reactions**	**AM2120**

Not For Medical Diagnostic Use. The system contains sufficient reagents for 200 reactions at 20µl. Includes:

- 2 × 100µl N2 & PMMoV Primer/Probe/IAC Mix, 20X
- 2 × 1,000µl GoTaq® Enviro Master Mix, 2X
- 1 × 100µl GoScript™ Enzyme Mix
- 2 × 1.25ml Nuclease-Free Water
- 1 × 100µl SARS-CoV-2 (N+E) RNA, 4 × 10^6 copies/µl
- 1 × 100µl PMMoV RNA, 4 × 10^6 copies/µl

2. Product Components and Storage Conditions (continued)

PRODUCT	SIZE	CAT.#
GoTaq® Enviro Wastewater SARS-CoV-2 System, E	**200 reactions**	**AM2130**

Not For Medical Diagnostic Use. The system contains sufficient reagents for 200 reactions at 20µl. Includes:

- 2 × 100µl E & PMMoV Primer/Probe/IAC Mix, 20X
- 2 × 1,000µl GoTaq® Enviro Master Mix, 2X
- 1 × 100µl GoScript™ Enzyme Mix
- 2 × 1.25ml Nuclease-Free Water
- 1 × 100µl SARS-CoV-2 (N+E) RNA, 4×10^6 copies/µl
- 1 × 100µl PMMoV RNA, 4×10^6 copies/µl

Storage Conditions: Store all components of the GoTaq® Enviro Wastewater SARS-CoV-2 Systems at –30°C to –10°C. Limit freeze-thaws to five cycles or less. Store the 20X Primer/Probe/IPC Mixes protected from light.

3. General Considerations

GoTaq® Enviro Wastewater SARS-CoV-2 Systems are sensitive; take precautions to minimize contamination. We recommend storing the reagents separately from RNA/TNA (total nucleic acid) samples. We also recommend using clean designated work areas and separate pipettes for pre- and post-amplification steps to minimize the potential for cross contamination between RNA samples and to prevent carryover of nucleic acid from one run to the next. Wear a lab coat and protective eyewear. Wear gloves and change them often. Prevent contamination by using aerosol-resistant pipette tips. Always include a no-template control (NTC) reaction to detect contamination. We recommend performing NTC reactions in triplicate.

! Do not unseal reaction plates after amplification is complete. Unsealing the plates increases the risk of contaminating subsequent reactions with amplified product.

Materials to Be Supplied by User

- sterile aerosol-resistant barrier pipette tips
- pipettes dedicated to pre-amplification work
- 1.5ml tubes to prepare the reaction mixes
- 0.5ml low-bind tubes (e.g., Eppendorf Cat.# 022431005) to prepare the standard dilutions
- qPCR plates or strip tubes with caps
- qPCR thermocycler (FAM™, HEX™, Cy®5 channels; ROX™ channel if reference dye required; see Section 8.B)

3.A. System Usage

The GoTaq® Enviro Wastewater SARS-CoV-2 Systems are RT-qPCR kits designed for detecting SARS-CoV-2 genetic signal from wastewater samples that have been preprocessed. Processing includes viral concentration and purification of nucleic acid. The purified nucleic acid is then used for RT-qPCR.

Viral concentration and purification can be achieved by using one of the following kits:

- Wizard® Enviro TNA Kit (Cat.# A2991)
- Maxwell® RSC Enviro TNA Kit (Cat.# AS1831)

Alternative viral concentration methods can also be used, such as PEG 8000/NaCl precipitation, membrane filtration, centrifugal ultrafiltration, skimmed milk flocculation or other methods. Nucleic acid purification can be performed on the concentrated viral material using manual or automated systems.

4. GoTaq® Enviro Wastewater SARS-CoV-2 Systems Protocol

Note: To avoid contamination of samples with external sources of PCR templates, perform all steps with aerosol-resistant pipette tips.

4.A. Preparing Standard Curve Dilutions for SARS-CoV-2 (N+E) and PMMoV RNAs

1. Thaw the SARS-CoV-2 (N+E) RNA, 4×10^6 copies/µl, and PMMoV RNA, 4×10^6 copies/µl. To avoid long exposure to ambient temperature, place reagents and standards on ice after thawing.
2. Combine and dilute the SARS-CoV-2 (N+E) and PMMoV RNAs (4×10^6 copies/µl) 100-fold by adding 2µl of each RNA to 196µl of Nuclease-Free Water, for a final concentration of 4×10^4 copies/µl.
3. Prepare serial tenfold dilutions in low-binding 0.5ml tubes. For example, combine 5µl of RNA with 45µl of Nuclease-Free Water to obtain the following standard curve dilutions (4×10^4–4 copies/µl; see Table 1 and Figure 1). Vortex each dilution for 3–5 seconds prior to removing an aliquot for the next dilution. Change pipette tips between dilutions.

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

Tube (Figure 1)	SARS-CoV-2 (N+E) and PMMoV RNA (copies/µl)	Copies/Well (5µl sample/ 20µl reaction)	Copies/Well (4µl sample/ 10µl reaction)
A	4×10^4	2×10^5	1.6×10^5
B	4×10^3	2×10^4	1.6×10^4
C	4×10^2	2×10^3	1.6×10^3
D	40	2×10^2	1.6×10^2
E	4	20	16

4.A. Preparing Standard Curve Dilutions for SARS-CoV-2 (N+E) and PMMoV RNAs (continued)

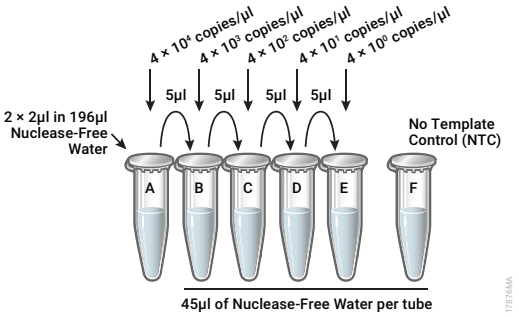

Figure 1. Dilution scheme for combined SARS-CoV-2 (N+E) and PMMoV RNA standards.

4.B. Preparing the RT-qPCR Amplification Mix (20µl Reaction Volume)

We recommend preparing three RT-qPCR technical replicates for increased statistical power.

1. Vigorously vortex the GoTaq® Enviro Master Mix for 30–60 seconds to ensure homogeneity before use. Briefly centrifuge to collect contents at the bottom of the tube.
2. Determine the number of reaction wells needed. This should include reactions for combined SARS-CoV-2 (N+E) and PMMoV RNA standards and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach consumes a small additional amount of each reagent, it ensures that enough RT-qPCR amplification mix will be available for all samples. It also ensures that each reaction contains the same RT-qPCR amplification mix.

   Table 2. Reaction Mix Worksheet for 20µl Reaction Volume.

RT-qPCR Amplification Mix	Volume per Reaction (X)	Number of Reactions (n)	Final Volume (X × n)
GoTaq® Enviro Master Mix, 2X	10µl		
GoScript™ Enzyme Mix	0.4µl		
Primer/Probe/IAC Mix, 20X	1µl		
Nuclease-Free Water	3.6µl		

3. Assemble the reaction mix by combining the GoTaq® Enviro Master Mix, GoScript™ Enzyme Mix, 20X Primer/Probe/IAC Mix and Nuclease-Free Water calculated in Step 2.
4. Pipette 15µl of RT-qPCR amplification mix into wells of 96-well qPCR plates.
5. Add 5µl of extracted nucleic acid, combined SARS-CoV-2 (N+E) and PMMoV RNA standards or Nuclease-Free Water for NTC. The final reaction volume should be 20µl.
6. Seal and centrifuge the plate at approximately 300 × *g* for 1 minute to ensure all liquid is collected at the bottom of the plate wells. Protect from extended light exposure and elevated temperatures before cycling. The samples are now ready for thermal cycling.

4.C. Preparing the RT-qPCR Amplification Mix (10µl Reaction Volume)

The systems are also amenable to 10µl reaction volumes.

1. Vigorously vortex the GoTaq® Enviro Master Mix for 30–60 seconds to ensure homogeneity before use. Briefly centrifuge to collect contents at the bottom of the tube.
2. Determine the number of reaction wells needed. This should include reactions for the combined SARS-CoV-2 (N+E) and PMMoV RNA standards and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach consumes a small additional amount of each reagent, it ensures that enough RT-qPCR amplification mix will be available for all samples. It also ensures that each reaction contains the same RT-qPCR amplification mix.

   Table 3. Reaction Mix Worksheet for 10µl Reaction Volume.

RT-qPCR Amplification Mix	Volume per Reaction (X)	Number of Reactions (n)	Final Volume (X × n)
GoTaq® Enviro Master Mix, 2X	5µl		
GoScript™ Enzyme Mix	0.2µl		
Primer/Probe/IAC Mix, 20X	0.5µl		
Nuclease-Free Water	0.3µl		

3. Assemble the reaction mix by combining the GoTaq® Enviro Master Mix, GoScript™ Enzyme Mix, 20X Primer/Probe/IAC Mix and Nuclease-Free Water calculated in Step 2.
4. Pipette 6µl of RT-qPCR amplification mix into wells of 96-well qPCR plates.
5. Add 4µl of extracted nucleic acid, combined SARS-CoV-2 (N+E) and PMMoV RNA standards or Nuclease-Free Water for NTC. The final reaction volume should be 10µl.
6. Seal and centrifuge the plate at approximately 300 × *g* for 1 minute to ensure all liquid is collected at the bottom of the plate wells. Protect from extended light exposure and elevated temperatures before cycling. The samples are now ready for thermal cycling.

4.C. Preparing the RT-qPCR Amplification Mix (10µl Reaction Volume; continued)

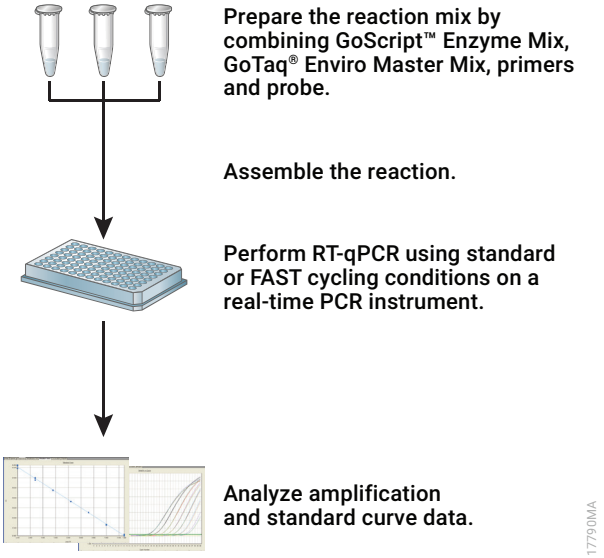

Figure 2. An overview of the GoTaq® Enviro Wastewater SARS-CoV-2 Systems protocol.

5. Thermal Cycling

The PCR cycling parameters and instrument settings shown here are provided as guidelines and can be modified as necessary for optimal results.

Standard Cycling Conditions

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	

FAST Cycling Conditions

Step	Temperature (°C)	Time	Number of Cycles
Reverse transcription	45	15 minutes	1
RT inactivation/GoTaq® activation	95	2 minutes	1
Denaturation	95	3 seconds	40
Annealing/extension	62	30 seconds	

Collect data from the following fluorescence channels at the end of each 62°C annealing/extension step. Performing >40 PCR cycles is **not** recommended as it may generate nonspecific amplification products.

Fluorophores	Target
FAM™	N1, N2, E (SARS-CoV-2)
CAL Fluor® 560/HEX™/JOE™/VIC®	Internal Amplification Control
ROX™/CXR	Reference Dye
Quasar® 670/Cy®5	PMMoV

Dispose of PCR plates as biohazardous waste per your institutional guidelines. To avoid DNA contamination of your lab space and subsequent samples, do not open the PCR plates after completing amplification and data collection.

5. Thermal Cycling (continued)

	SARS-CoV-2 (N+E) and PMMoV RNA Standards or NTC			Nucleic Acid Purified from Wastewater								
	1	2	3	4	5	6	7	8	9	10	11	12
A	2×10^5	2×10^5	2×10^5	Sample 1	Sample 1	Sample 1	Sample 9	Sample 9	Sample 9	Sample 17	Sample 17	Sample 17
B	2×10^4	2×10^4	2×10^4	Sample 2	Sample 2	Sample 2	Sample 10	Sample 10	Sample 10	Sample 18	Sample 18	Sample 18
C	2×10^3	2×10^3	2×10^3	Sample 3	Sample 3	Sample 3	Sample 11	Sample 11	Sample 11	Sample 19	Sample 19	Sample 19
D	2×10^2	2×10^2	2×10^2	Sample 4	Sample 4	Sample 4	Sample 12	Sample 12	Sample 12	Sample 20	Sample 20	Sample 20
E	20	20	20	Sample 5	Sample 5	Sample 5	Sample 13	Sample 13	Sample 13	Sample 21	Sample 21	Sample 21
F	NTC	NTC	NTC	Sample 6	Sample 6	Sample 6	Sample 14	Sample 14	Sample 14	Sample 22	Sample 22	Sample 22
G				Sample 7	Sample 7	Sample 7	Sample 15	Sample 15	Sample 15	Sample 23	Sample 23	Sample 23
H				Sample 8	Sample 8	Sample 8	Sample 16	Sample 16	Sample 16	Sample 24	Sample 24	Sample 24

Figure 3. Example plate layout for one target (N1, N2 or E), plus standard RNAs and NTCs. Note: For the 10µl reaction size refer to Table 1, Section 4.A for accurate copy numbers per reaction in the standard curve wells.

6. Data Analysis and Interpretation

6.A. Evaluate qPCR Assay Standard Curves (FAM [SARS-CoV-2]/Cy5 [PMMoV])

Common qPCR analysis software packages apply a linear regression to the standard dilution series data and calculate the best fit of the standard curve using $y = mx + b$, where $x = \text{Log}_{10}$ concentration; $y = C_q/C_t$; m = slope. r^2 measures goodness of fit to the regressed line and m is a measure of efficiency, where m= −3.3 indicates 100% PCR efficiency (i.e., amplification product is doubled at each cycle). The y intercept (b in the equation) is the y value C_q at x = 0. For example, b corresponds to the C_q value for a sample with a concentration of 1 copy/reaction ($\text{Log}_{10}(1) = 0$).

In general, the standard curve for each PCR target has an average slope (m) in the range of −3.0 to −3.7 (corresponding to a qPCR efficiency of 86%<E<115%) and an r^2 value >0.970. We recommend monitoring y-intercept values for any significant changes from run to run.

6.B. Analyze Internal Amplification Control Signal (HEX/CAL Fluor 560/VIC/JOE)

The IAC can be used to evaluate overall performance of the SARS-CoV-2 RT-qPCR amplification reaction and to detect DNA polymerase and/or reverse transcriptase inhibition. The probe used is a dual-labeled probe (CAL Fluor® 560/BHQ1). Depending on the qPCR instrument used, HEX, JOE, VIC and CAL Fluor® 560 channels can be used to record the amplification signal. Depending on the qPCR instrument and analysis software used, the IAC C_t should fall in the range of 20–30 for the NTC reactions.

If the IAC C_t in a sample well is shifted significantly ($C_t \geq 2$) compared to NTC well, PCR inhibitors are present in the experimental sample, and results should be considered qualitative and not quantitative. Repeat the purification or clean-up of nucleic acid if necessary. If a sample yields no detectable amplification for SARS-CoV-2 but exhibits IAC amplification (C_t = 20–30) and PMMoV amplification (C_t = 20–40), SARS-CoV-2 is not detectable with this system. If the IAC fails to amplify or the IAC C_t is shifted > 3 C_t compared to NTC wells, no conclusions can be made about the absence of SARS-CoV-2 genetic material in a sample. Results can be considered invalid. See Table 4 for examples.

IAC can fail to amplify if assay is setup incorrectly.

6.C. Analyze Process Control Signal, PMMoV (Cy5/Quasar 670)

Wastewater samples typically exhibit PMMoV fluorescence growth curves that cross the threshold at <40 cycles.

If a sample yields no detectable amplification for SARS-CoV-2 but exhibits IAC amplification (C_t = 20–30), and PMMoV amplification (C_t = 20–40), SARS-CoV-2 is not detectable with this system.

Failure to detect PMMoV in wastewater samples may indicate:

- improper extraction of nucleic acid from samples resulting in loss of RNA, RNA degradation or both
- inhibition of reverse transcriptase, DNA polymerase or both by inhibitors in the sample
- absence of sufficient nucleic acid due to poor collection or pasteurization of sample
- improper assay set up and execution
- reagent or equipment malfunction

If the PMMoV reaction (Cy®5 channel) is negative, IAC is positive and SARS-CoV-2 N1 or N2 or E are positive, the result can be considered valid because PMMoV negativity may reflect a low PMMoV viral load.

If all SARS-CoV-2 markers, PMMoV (process control) and internal amplification control (IAC) are negative for the specimen, the results are invalid. If residual sample is available, repeat the extraction procedure and retest. If all markers remain negative after retesting, report the results as invalid. A new specimen should be collected if possible.

6.D. No-Template Control (NTC)

For an NTC, use Nuclease-Free Water in the RT-qPCR instead of a nucleic acid-containing sample or RNA standards. NTC samples should produce amplification curves for the IAC in the HEX™ channel. Sample contamination is indicated if FAM™ or Cy®5 NTC reaction channels exhibit fluorescence curve with C_t value indicating copy number greater than the limit of quantification (LOQ). LOQ for the assay is 20 copies per reaction for SARS-CoV-2 genetic signal (in the FAM channel: N1, N2 and E, respectively).

6.E. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD is defined as the lowest amount of analyte in a sample that can be detected with 95% probability. The LOD for these assays is 8 copies of nucleic acid per reaction.

LOQ is defined as the lowest amount of analyte in a sample that can be quantitatively determined with less than 25% coefficient of variance (8–10).

In Nuclease-Free Water (reference matrix) spiked with SARS-CoV-2 RNA, the LOQ of the assay is 20 copies per reaction. If SARS-CoV-2 genetic signal amplification appears after the signal for the LOQ, the amount of SARS-CoV-2 in the sample cannot be determined with certainty.

6.F. Interpreting Wastewater Signal Profiles

Table 4. Interpreting Wastewater Results.

SARS-CoV-2				PMMoV Process Control (Cy5)	Internal Amplification Control (HEX)	
N1 (FAM)	N2 (FAM)	E (FAM)	NTC (FAM)			Result
Any one or more is positive			–	+/–	+/–	SARS-CoV-2 Detected
–	–	–	–	+	+	SARS-CoV-2 Not Detected
Any one or more is positive			+	+/–	+/–	Invalid (False Positive)
–	–	–	–	+/–	–	Invalid (False Negative)

6.G. Calculating Viral Nucleic Acid

The following formula can be applied to quantitate the amount of SARS-CoV-2 nucleic acid in a sample:

$$\text{Viral genome (copies/liter)} = \frac{\text{Copies in RT-qPCR} \times 1{,}000}{\text{Volume of nucleic acid extract used in RT-qPCR (ml)*} \times \text{Concentration factor}}$$

*If 5µl of nucleic acid extract is used in RT-qPCR, the value in ml is 0.005.

$$\text{Concentration factor} = \frac{\text{Wastewater sample volume used (ml)}}{\text{Volume of nucleic acid extracted (ml)}}$$

6.H. Normalization with PMMoV

Quantitation of PMMoV viral genome copies can be performed using the same approach as for SARS-CoV-2 using the PMMoV RNA Quant Standard.

Changes in SARS-CoV-2 levels can be analyzed relative to the PMMoV levels by using this formula:

$$\text{Relative SARS-CoV-2 signal} = \frac{\text{SARS-CoV-2 signal (copies/liter)}}{\text{PMMoV signal (copies/liter)}}$$

7. Specificity Testing

Wastewater TNA isolates contain abundant nucleic acid originating from various bacterial and viral species. The GoTaq® Enviro Wastewater SARS-CoV-2 Systems were carefully designed to amplify only the designated SARS-CoV-2 genomic target.

Table 5. Specificity of the GoTaq® Enviro Wastewater SARS-CoV-2 Systems When Tested Against Respiratory Pathogens.

Pathogen	N1	N2	E
SARS-CoV-2	+	+	+
OC43	–	–	–
229E	–	–	–
HKU1	–	–	–
NL63	–	–	–
Influenza A	–	–	–
RSV	–	–	–
L. pneumophila	–	–	–
P. aeruginosa	–	–	–

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8. Appendix

8.A. References

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8.B. qPCR Instruments and Reference Dye Requirements

Instruments that do not require supplemental reference dye:

- Bio-Rad CFX96 Real-Time PCR Detection System
- Bio-Rad/MJ Research Chromo4™ Real-Time Detector
- Bio-Rad iCycler iQ® and iQ®5 Real-Time PCR Detection Systems
- Roche LightCycler® 480 Real-Time PCR System
- MyGo Pro IT-IS

Instruments that require low levels (30nM) of reference dye:

- Applied Biosystems® 7500 and 7500 FAST Real-Time PCR System
- Applied Biosystems® QuantStudio® Real Time PCR Systems
- Applied Biosystems® ViiA® 7 Real-Time PCR System
- Stratagene/Agilent Mx3000P® and Mx3005P® Real-Time PCR Systems
- Stratagene/Agilent Mx4000® Multiplex Quantitative PCR System

Instruments that require high levels (500nM) of reference dye:

- Applied Biosystems® StepOne™ and StepOnePlus™ Real-Time PCR Systems
- Applied Biosystems® 7300 and 7900HT Real-Time PCR System

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8.C. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. Email: **techserv@promega.com**

Symptoms	Causes and Comments
Internal Amplification Control (IAC) C_t in a sample well is shifted significantly (C_t ≥2) compared to NTC well	PCR inhibitors are present in the experimental sample and results should be considered qualitative and not quantitative. Repeat the purification or clean-up of nucleic acid if necessary.
	If the IAC fails to amplify or the IAC C_t is shifted >3 C_t compared to NTC wells, no conclusions can be made about the absence of genetic material in a sample. Results can be considered invalid.
	IAC can fail to amplify if the assay is set up incorrectly.
Failure to detect qPCR signal	Improper nucleic acid extraction from samples, resulting in loss of RNA, RNA degradation or both.
	Inhibition of reverse transcriptase and/or DNA polymerase by inhibitors in the sample. Absence of sufficient nucleic acid due to poor collection or pasteurization of sample.
	Absence of sufficient nucleic acid due to poor collection or pasteurization of sample.
	Improper assay set up or execution. Reagent or equipment malfunction.
Low yield of RT-qPCR product	RNA degradation. Always use nuclease-free, commercially autoclaved reaction tubes, sterile aerosol resistant tips and gloves. Ensure that reagents, tubes and tips are kept RNase-free by using sterile technique.
	Poor primer design. If the reaction products appear to be entirely primer artifacts, the reaction may not have amplified the desired RT-PCR product because of primer-primer interactions. Make sure the primers are not self-complementary. Check the length and melting temperature of the PCR primers.
	Extension time was too brief for amplicon length. To minimize interactive effects of reverse transcriptase and thermophilic DNA polymerase, design the thermal cycling program with a longer extension time in each cycle. Begin with 1 minute per kilobase per cycle and increase to 2 minutes or more if necessary.

8.D. Related Products

Amplification Systems and Accessories

Product	Size	Cat.#
SARS-CoV-2 (N+E) dsDNA Quant Standard	100µl	AM2060
PMMoV RNA Quant Standard	100µl	AM2070
SARS-CoV-2 (N+E) RNA Quant Standard	100µl	AM2050
GoTaq® Enviro qPCR System*	200 reactions	AM2000
	1,000 reactions	AM2001
GoTaq® Enviro RT-qPCR System*	200 reactions	AM2010
	1,000 reactions	AM2011
IPC qPCR Inhibition Control Assay, CAL Fluor® 560*	100 reactions	AM2030
IAC RT-qPCR Inhibition Control Assay, CAL Fluor® 560*	100 reactions	AM2040
GoScript™ Reverse Transcriptase	100 reactions	A5003
	500 reactions	A5004
RNasin® Plus RNase Inhibitor	2,500u	N2611
	10,000u	N2615
Set of dATP, dCTP, dGTP, dUTP	10µmol each	U1335
	40µmol each	U1245
RQ1 RNase-Free DNase	1,000u	M6101
$MgCl_2$	1.5ml	A3511
Nuclease-Free Water	50ml	P1193

*For Research Use Only. Not for use in diagnostic procedures.
Not For Medical Diagnostics Use.

Manual Nucleic Acid Purification Systems and Reagents

Product	Size	Cat.#
Wizard® Enviro TNA Kit	25 preps	A2991
Vac-Man® 96 Vacuum Manifold	1 each	A2291
Eluator™ Vacuum Elution Device	4 each	A1071
PEG 8000, Molecular Biology Grade	500g	V3011
Sodium Chloride, Molecular Biology Grade	1kg	H5273

Promega

8.D. Related Products (continued)

Automated RNA Purification

Product	Size	Cat.#
Maxwell® RSC Enviro TNA Kit	48 preps	AS1831
Maxwell® RSC PureFood GMO and Authentication Kit	48 preps	AS1600

9. Summary of Changes

The following change was made to the 4/24 revision of this document:

1. Added Troubleshooting, Section 8.C.
2. Minor text edits were made.