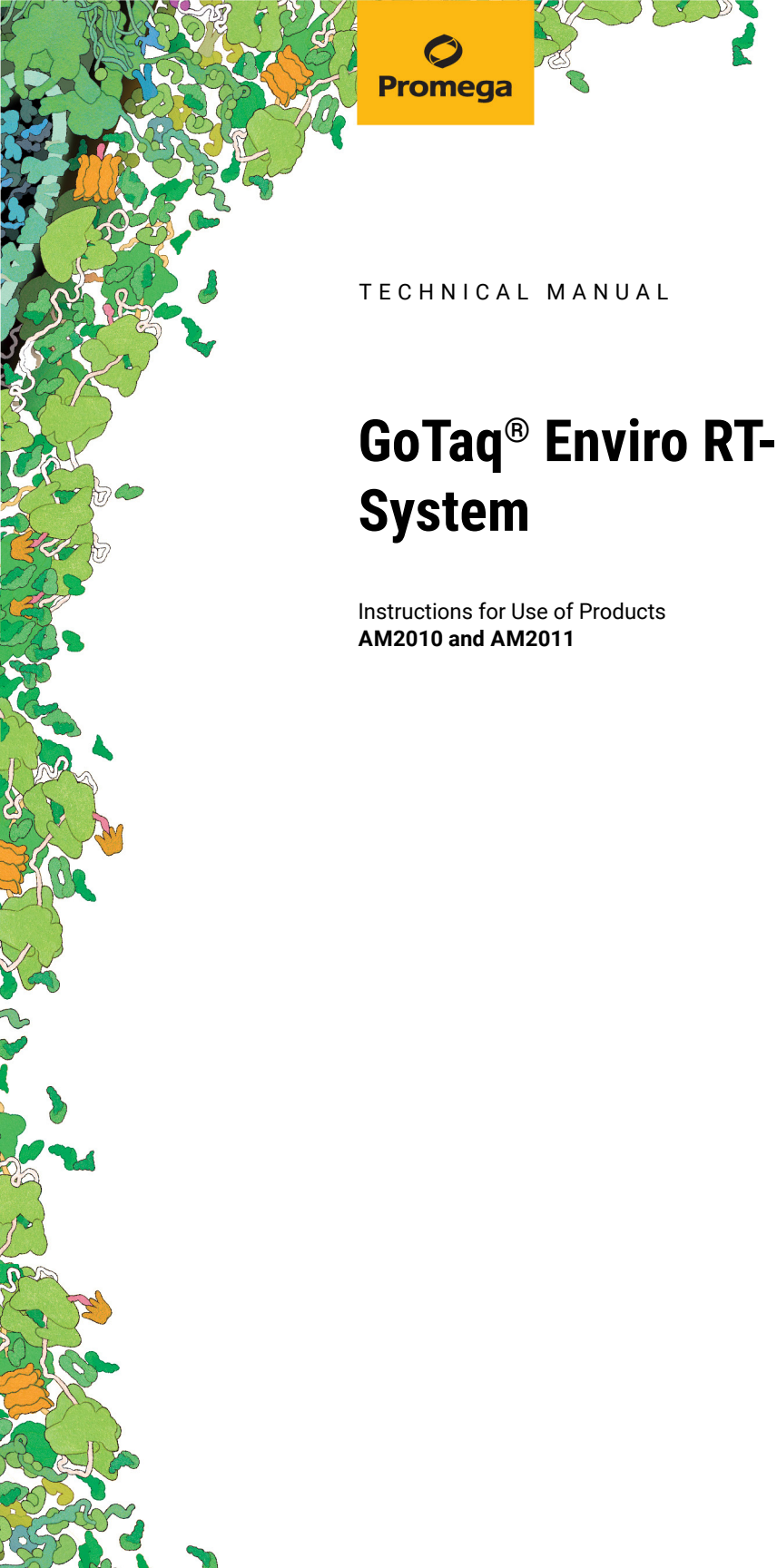

TECHNICAL MANUAL

GoTaq® Enviro RT-qPCR System

Instructions for Use of Products
AM2010 and AM2011

Revised 8/23
TM659

GoTaq® Enviro RT-qPCR System

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

1. Description

The GoTaq® Enviro RT-qPCR System was developed for performing quantitative PCR assays using hydrolysis probes for real-time amplicon detection. The RT-qPCR System is optimized for amplifying RNA targets from environmental samples that may contain PCR and RT-qPCR inhibitors, such as humic acid, tannic acid and other compounds. The system detects and quantifies relative RNA levels using a one-step RT-qPCR method, combining GoScript™ Reverse Transcriptase and GoTaq® Enviro Master Mix in a real-time amplification reaction. An overview of the protocol is shown in Figure 1.

The GoScript™ Enzyme Mix for 1-Step RT-qPCR (50X) combines optimized amounts of GoScript™ Reverse Transcriptase, RNasin® Plus RNase Inhibitor and additives to enhance single-step reactions.

The GoTaq® Enviro Master Mix is provided as a ready-to-use, stabilized 2X formulation that includes all components for qPCR, including GoTaq® Hot Start Polymerase, $MgCl_2$, dNTPs and a proprietary reaction buffer, but does not include template, primers and probe. The master mix does not contain a reference dye. A separate tube of carboxy-X- rhodamine (CXR) reference dye is included with this system for adding reference dye to amplification reactions if desired. Section 3.C includes details on instruments and requirements for CXR reference dye.

The GoTaq® Enviro RT-qPCR System provides resistance to a wide range of PCR inhibitors commonly found in environmental samples. This formulation uses antibody-mediated hot-start chemistry for performing reaction setup at room temperature. The master mix also employs rapid hot-start activation and processive enzymes, making it compatible with both standard and fast instrument cycling programs.

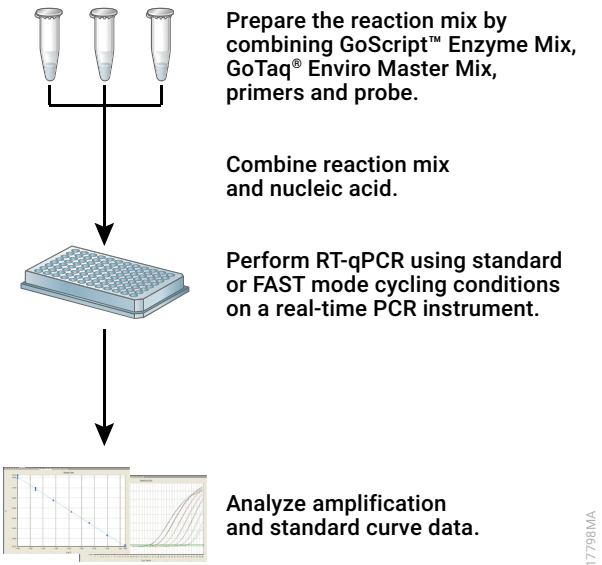

Figure 1. Overview of the GoTaq® Enviro RT-qPCR System protocol.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
GoTaq® Enviro RT-qPCR System	**200 reactions**	**AM2010**

For Research Use Only. Not for use in diagnostic procedures. Each system contains sufficient reagents for 200 × 20µl reactions or 400 × 10µl reactions. Includes:

- 2 × 1ml GoTaq® Enviro Master Mix, 2X
- 1 × 100µl GoScript™ Enzyme Mix, 50X
- 1 × 100µl CXR Reference Dye, 30µM
- 2 × 1.25ml Nuclease-Free Water

PRODUCT	SIZE	CAT.#
GoTaq® Enviro RT-qPCR System	**1,000 reactions**	**AM2011**

For Research Use Only. Not for use in diagnostic procedures. Each system contains sufficient reagents for 1,000 × 20µl reactions or 2,000 × 10µl reactions. Includes:

- 10 × 1ml GoTaq® Enviro Master Mix, 2X
- 5 × 100µl GoScript™ Enzyme Mix, 50X
- 1 × 100µl CXR Reference Dye, 30µM
- 1 × 13ml Nuclease-Free Water

Storage Conditions: Store all components at –30°C to –10°C. Protect CXR Reference Dye, 30µM, from light at all times. For best results, mix thawed solutions gently to minimize aeration and foaming, and keep on ice. For short-term storage and frequent use, store GoTaq® Enviro Master Mix, 2X, at +2°C to +10°C for up to 3 months, protected from light. Do not freeze-thaw the GoTaq® Enviro Master Mix, 2X, more than 5 times.

3. General Considerations

3.A. Preventing Contamination

We recommend the following precautions to prevent contamination:

- Use designated work areas and pipettes for pre- and post-amplification steps to minimize the potential for cross contamination between samples and prevent carryover of nucleic acids from one experiment to the next.
- Wear gloves and change them often.
- Do not open the reaction plate or strip wells after amplification is complete. Opening the reaction plate or strip wells increases the risk of contaminating subsequent reactions with the amplified product.
- Use aerosol-resistant barrier pipette tips.

Promega

3.B. qPCR Primers and Probes

The concentrations of primers and probes should be optimized for each primer/probe combination. For gene expression assays, primer and probe concentrations may need to be adjusted based on target abundance and/or primer specificity. We recommend a concentration of 900nM for PCR primers and 250nM for the hydrolysis probe as a starting point.

Concentrations of PCR primers can range from 200nM to 1µM, while probe concentration can range from 100nM to 300nM; titrations should be performed to ensure optimal results.

We recommend preparing and storing the PCR primers and hydrolysis probes as 20X solutions.

3.C. CXR Reference Dye

The GoTaq® Enviro Master Mix, 2X, does not contain a reference dye; however, a separate tube of carboxy-X-rhodamine (CXR) Reference Dye is included with this system, allowing the addition of reference dye if desired. Adding the reference dye will help maximize effectiveness of the GoTaq® Enviro Master Mix, 2X, when used with real-time PCR instruments that allow normalization. The CXR Reference Dye, 30µM, has the same spectral properties as ROX™ dye. The CXR Reference Dye is provided at a concentration of 30µM.

Some instrumentation is designed to normalize with a low concentration of ROX™ reference dye. We recommend that the CXR Reference Dye, 30µM, be added to a final concentration of 30nM for instruments that recommend a low level of ROX™ dye. Other instruments require ROX™ at a high concentration for normalization. We recommend that the CXR Reference Dye, 30µM, be added to a final concentration of 500nM for instruments that recommend a high level of ROX™ dye.

Recommended CXR Reference Dye, 30µM, levels for various qPCR instruments are listed below. Directions for supplementing the GoTaq® Enviro Master Mix, 2X, with CXR Reference Dye, 30µM, are included in Section 4.A.

Instruments that do not require supplemental reference dye:

- Bio-Rad CFX96 Real-Time PCR Detection System
- Bio-Rad DNA Engine Opticon® and Opticon® 2 Real-Time PCR Detection Systems
- Bio-Rad/MJ Research Chromo4™ Real-Time Detector
- Bio-Rad iCycler iQ® and iQ®5 Real-Time PCR Detection Systems
- Bio-Rad MyiQ™ Real-Time PCR Detection System
- Roche LightCycler® 480 Real-Time PCR System
- Eppendorf Mastercycler® ep realplex Real-Time PCR System

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

4. GoTaq® Enviro RT-qPCR System Protocol

Materials to Be Supplied by the User

- real-time PCR instrument and related consumables (i.e., optical-grade PCR plates and appropriate well caps or sealing film)
- sterile, aerosol-resistant barrier pipette tips
- nuclease-free pipettors dedicated to pre-amplification work
- RNA/total nucleic acid (TNA) template
- qPCR primers and probe

4.A. Optional: Adding CXR Reference Dye, 30µM, to GoTaq® Enviro Master Mix, 2X

Some real-time PCR instruments require addition of CXR Reference Dye; see Section 3.C. If you wish to add CXR Reference Dye to your amplification reactions, we recommend adding an aliquot of concentrated CXR Reference Dye, 30µM, to the 1ml tube of the GoTaq® Enviro Master Mix, 2X. Depending on your instrument, the CXR Reference Dye, 30µM, can be added to either the low dye (30nM) or high dye (500nM) concentration (Section 3.C).

1. Thaw the GoTaq® Enviro Master Mix, 2X, CXR Reference Dye, 30µM, and Nuclease-Free Water at ambient temperature.
2. Vigorously vortex the GoTaq® Enviro Master Mix, 2X, for 30–60 seconds to ensure homogeneity before use. Briefly centrifuge to collect contents at the bottom of the tube.
3. Add CXR Reference Dye, 30µM, to the GoTaq® Enviro Master Mix, 2X:
    a. **For high-dye instruments:** Add 33.4µl of CXR Reference Dye, 30µM, to the 1ml tube of GoTaq® Enviro Master Mix, 2X.
    b. **For low-dye instruments:** Add 2µl of CXR Reference Dye, 30µM, to the 1ml tube of GoTaq® Enviro Master Mix, 2X.
4. Vortex for 3–5 seconds to mix.
5. Mark the tube to indicate that you have performed this step. Store the GoTaq® Enviro Master Mix, 2X, with CXR Reference Dye, at –30°C to –10°C and protect from light at all times.

    Note: Aliquot the GoTaq® Enviro Master Mix, 2X, combined with CXR Reference Dye if more than 5 freeze-thaw cycles will occur before used completely.

4.B. Assembling the GoTaq® Enviro RT-qPCR Amplification Mix

The GoTaq® Enviro Master Mix, 2X, uses a hot-start chemistry for performing reaction setup at room temperature. The final reaction volume in this protocol is 20µl. The volumes given here may be scaled for larger or smaller reaction volumes.

1. Thaw the GoTaq® Enviro Master Mix, 2X, and vigorously vortex 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 reactions to be set up, including negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach requires using a small additional amount of extra reagent, it ensures that you have enough reaction mix for all samples.
3. Prepare the amplification mix [minus the RNA or total nucleic acid (TNA) template] by combining the components as shown in the table below. The RNA/TNA template is added in Step 5. Vortex briefly to mix.

Component	Volume	Final Concentration
GoTaq® Enviro Master Mix, 2X	10µl	1X
GoScript™ Enzyme Mix	0.4µl	1X
Forward Primer (20X)	1µl	200nM–1µM
Reverse Primer (20X)	1µl	200nM–1µM
Hydrolysis-Probe (20X)	1µl	100–300nM
Template RNA/TNA	2–5µl	≤250ng
Nuclease-Free Water to a final volume of	**20µl**	—

Note: The concentrations of primers and hydrolysis probe should be optimized for each primer combination.

4. Add the appropriate volume of amplification mix (without the template) to each PCR tube or well of an optical-grade PCR plate.
5. Add the RNA/TNA template (or water for the no-template control (NTC) reactions) to the appropriate wells of the reaction plate.
6. Seal the tubes or optical plate; spin briefly to collect the contents of the wells at the bottom by centrifuging the plate for 1 minute at 300 × *g*. Protect from extended light exposure or elevated temperatures before cycling. The samples are now ready for thermal cycling.

   Note: Assembled reaction plates can be stored protected from light at ambient temperatures for up to 4 hours.

5. Thermal Cycling

The cycling parameters below are offered as a guideline and may be modified as necessary for optimal results.

Standard Cycling Conditions

Step	Temperature	Time	Number of Cycles
Reverse transcription	45°C	15 minutes	1
Reverse transcriptase inactivation/ GoTaq® DNA Polymerase activation	95°C	2 minutes	1
Denaturation	95°C	15 seconds	40
Annealing and extension	60°C	1 minute	

FAST Cycling Conditions

Step	Temperature	Time	Number of Cycles
Reverse transcription	45°C	15 minutes	1
Reverse transcriptase inactivation/ GoTaq® DNA Polymerase activation	95°C	2 minutes	1
Denaturation	95°C	3 seconds	40
Annealing and extension	60°C	30 seconds	

Promega

6. Appendix

6.A. Inhibition Control Data

The GoTaq® Enviro RT-qPCR System is designed to tolerate PCR inhibitors that may be present in environmental samples. Inclusion of an exogenous internal control provides full confidence in qPCR results and data interpretation. The IAC RT-qPCR Inhibition Control Assay, CAL Fluor® 560 (Cat.# AM2040) contains primers, a hydrolysis probe (CAL Fluor® 560, which is compatible with HEX™ readout) and an exogenous DNA template.

Table 1 shows RT-qPCR reactions performed with the IAC RT-qPCR Inhibition Control Assay using either the GoTaq® Enviro RT-qPCR System (Cat.# AM2010) or GoTaq® Probe 1-Step RT-qPCR System (Cat.# A6120) with varying amounts of humic acid, a known PCR inhibitor. Nuclease-Free Water was used as a no-inhibitor control. No C_t indicates that PCR was completely inhibited by humic acid, while a ΔC_t of 0 indicates no inhibition by humic acid.

A shift in C_t value from the no-inhibitor control reflects the level of RT-qPCR inhibition.

$\Delta C_t = C_t$ [with Inhibitor] – C_t [no Inhibitor]

ΔC_t >2 represents significant inhibition of the reaction.

Table 1. GoTaq® Enviro RT-qPCR System Tolerates PCR Inhibitors.

	Humic Acid (ng/reaction)							
Assay	**125**	**62.5**	**31.25**	**15.63**	**7.81**	**3.91**	**1.95**	**0**
GoTaq® Enviro RT-qPCR System (ΔC_t)	10.58	5.81	2.42	1	0.42	0.2	0.02	0
GoTaq® Probe 1-Step RT-qPCR System (ΔC_t)	No C_t	No C_t	7.54	3.75	1.83	1.15	0.1	0

6.B. Troubleshooting

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

Symptoms	Causes and Comments
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.
	Isolate RNA in the presence of RNasin® Ribonuclease Inhibitor. Use RNasin® Ribonuclease Inhibitor to inhibit degradation of target during cDNA synthesis (20u/20µl reaction).
	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.
	Too few PCR cycles. To detect rare or difficult RNA targets by RT-PCR, increase the cycle number to 40 to maximize sensitivity.
	Wrong reaction tubes were used. Make sure to use thin-walled reaction tubes for optimal heat transfer during PCR. Use only sterile, nuclease-free commercially autoclaved tubes, strip tubes or plates for PCR. Autoclaving eliminates volatile contaminants that inhibit amplification.
	Reverse transcriptase effect on primer-dimer artifact synthesis in RT-PCR. Make sure to thoroughly heat-inactivate the reverse transcription reactions prior to use (6,7).

Promega

6.C. General qPCR References

1. Bustin, S.A. *et al*. (2009) The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem*. **55**, 611–22.
2. Dorak, M.T. (2009) Glossary of real-time PCR terms. This can be viewed online at: **www.dorak.info/genetics/glosrt.html**
3. Fleige, S. and Pfaffl, M.W. (2006) RNA integrity and the effect on the real-time qRT-PCR performance. *Mol. Aspects Med*. **27**, 126–39.
4. Lefever, S. *et al*. (2009) RDML: Structured language and reporting guidelines for real-time quantitative PCR data. *Nucleic Acids Res*. **37**, 2065–9.
5. Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods* **25**, 402–8.
6. Chumakov, K.M. (1994) Reverse transcriptase can inhibit PCR and stimulate primer-dimer formation. *PCR Methods Appl.* **4**, 62–4.
7. Sellner, L.N., Coelen, R.J. and Mackenzie, J.S. (1992) Reverse transcriptase inhibits Taq polymerase activity. *Nucleic Acids Res.* **20**, 1487–90.

6.D. Related Products

Real-Time PCR and RT-PCR Reagents

Product	Size	Cat.#
GoTaq® qPCR Master Mix*	5ml	A6001
IPC qPCR Inhibition Control Assay, CAL Fluor® 560*	100 reactions	AM2030
GoTaq® Enviro qPCR System*	200 reactions	AM2000
	1,000 reactions	AM2001
GoTaq® 1-Step RT-qPCR Master Mix*	5ml	A6020
IAC RT-qPCR Inhibition Control Assay, CAL Fluor® 560*	100 reactions	AM2040
Nuclease-Free Water	50ml	P1193
Set of dATP, dCTP, dGTP, dUTP	10µmol each	U1335
	40µmol each	U1245
CXR Reference Dye	100µl	C5411

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

RT-qPCR Systems and Standards

Product	Size	Cat.#
GoTaq® Enviro Wastewater SARS-CoV-2 System, N1/N2/E*	200 reactions	AM2100
GoTaq® Enviro Wastewater SARS-CoV-2 System, N1*	200 reactions	AM2110
GoTaq® Enviro Wastewater SARS-CoV-2 System, N2*	200 reactions	AM2120
GoTaq® Enviro Wastewater SARS-CoV-2 System, E*	200 reactions	AM2130
GoTaq® Enviro PMMoV Quant Kit, Quasar® 670	200 reactions	AM2140
PMMoV RNA Quant Standard	100µl	AM2070
SARS-CoV-2 (N+E) RNA Quant Standard	100µl	AM2050

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

Nucleic Acid Purification Kits and Accessories

Product	Size	Cat.#
Wizard® Enviro TNA Kit	25 preps	A2991
Maxwell® RSC Enviro TNA Extraction Kit	48 preps	AS1831
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
Maxwell® RSC PureFood GMO and Authentication Kit**	48 preps	AS1600
Maxwell® RSC PureFood Pathogen Kit**	48 preps	AS1660
Maxwell® RSC Plant DNA Kit	48 preps	AS1490
Maxwell® RSC Whole Blood DNA Kit*	48 preps	AS1520
Wizard® Genomic DNA Purification Kit*	100 isolations × 300µl	A1120
Maxwell® RSC simplyRNA Cells Kit**	48 preps	AS1390
Maxwell® RSC simplyRNA Blood Kit**	48 preps	AS1380
MagneSil® Total RNA Mini-Isolation System	4 plates	Z3351

*Additional sizes are available.

**Not for Medical Diagnostic Use.

7. Summary of Changes

The following changes were made to the 8/23 revision of this document:

1. Updated heading for Section 4.B.
2. Edited Step 2 in Section 4.A and Step 1 in Section 4.B.
3. Replaced document font.
4. Made minor text edits.