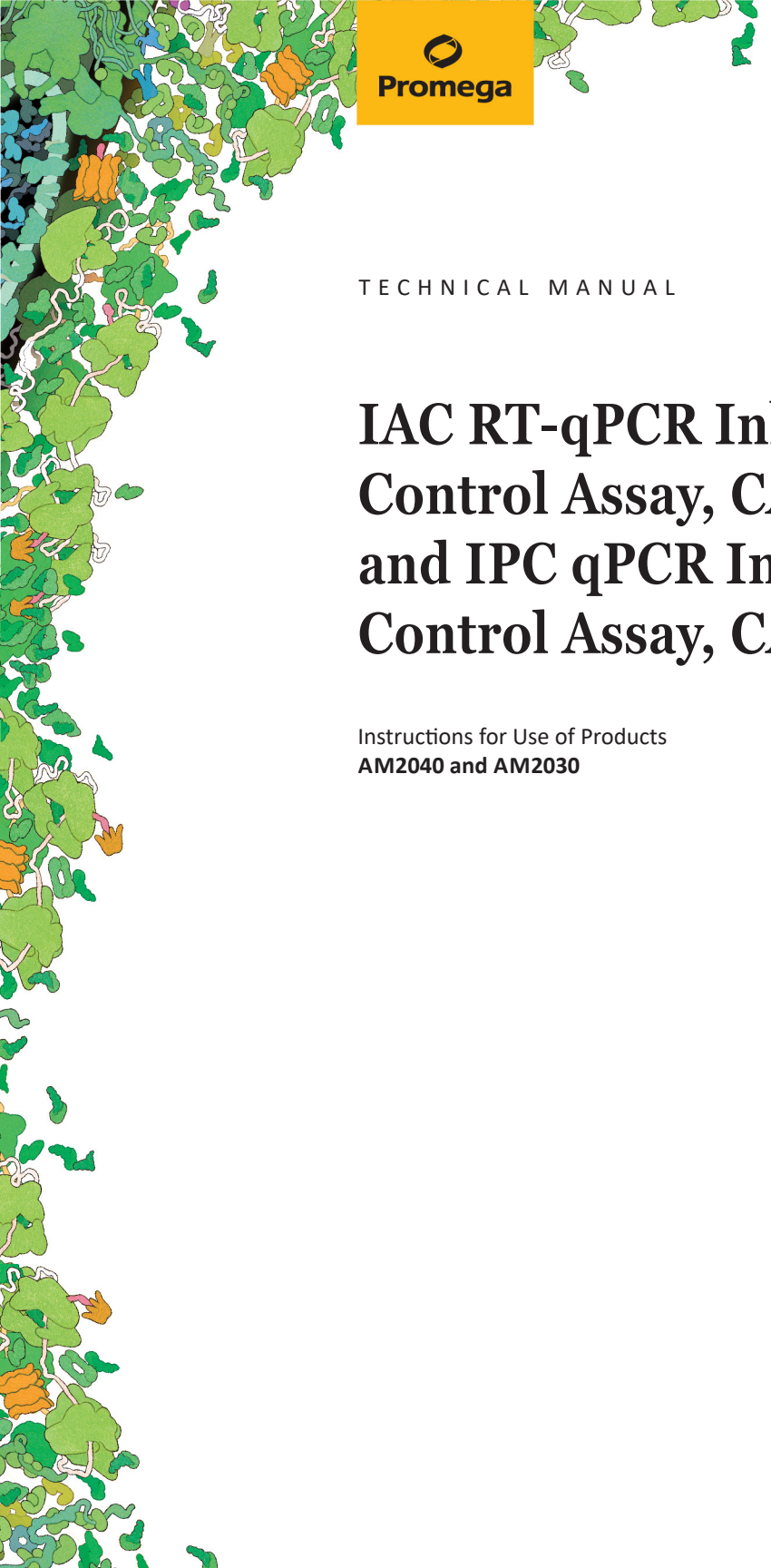

TECHNICAL MANUAL

IAC RT-qPCR Inhibition Control Assay, CAL Fluor® 560 and IPC qPCR Inhibition Control Assay, CAL Fluor® 560

Instructions for Use of Products
AM2040 and AM2030

11/21
TM657

IAC RT-qPCR Inhibition Control Assay, CAL Fluor® 560 and IPC qPCR Inhibition Control Assay, CAL Fluor® 560

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

Promega

1. Description

The use of an exogenous internal control in PCR provides full confidence in qPCR and RT-qPCR results and data interpretation. PCR or RT-PCR inhibitors, pipetting errors and thermocycler malfunctions are common causes of inconsistent results, and can be easily controlled for by including an Internal Positive Control (IPC) in the hydrolysis probe-based multiplexed amplification reaction. IPC provides information on DNA polymerase performance in a PCR reaction. IAC stands for Internal Amplification Control and provides information on reverse transcriptase and DNA polymerase performance in a one-step RT-qPCR reaction.

The IAC RT-qPCR Inhibition Control Assay, CAL Fluor® 560, contains primers (20X concentration, 6µM each), a hydrolysis probe labeled with CAL Fluor® 560 (20X concentration, 3µM) and an exogenous in vitro transcribed RNA template (20X concentration, 1pg/µl).

The IPC qPCR Inhibition Control Assay, CAL Fluor® 560, contains primers (20X concentration, 6µM each), a hydrolysis probe labeled with CAL Fluor® 560 (20X concentration, 3µM) and an exogenous linearized DNA template (20X concentration, 0.2pg/µl) that can be directly added to the RT-qPCR and qPCR reaction mixture.

The IAC RT-qPCR and IPC qPCR Inhibition Control Assays can be used with any DNA polymerase, including those found in GoTaq®, GoTaq® Enviro RT-qPCR and GoTaq® Enviro qPCR Systems.

Typically, Nuclease-Free Water is used as a no-template control (NTC). An increase in C_t value for the sample compared to NTC indicates the presence of inhibitors.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
IAC RT-qPCR Inhibition Control Assay, CAL Fluor® 560	**100 reactions**	**AM2040**

For Research Use Only. Not for use in diagnostic procedures. Each system contains sufficient reagents for 100 × 20µl reactions or 200 × 10µl reactions. Includes:

- 1 × 100µl IAC RT-qPCR Inhibition Control, CAL Fluor® 560, 20X
- 1 × 100µl CXR Reference Dye, 30µM

PRODUCT	SIZE	CAT.#
IPC qPCR Inhibition Control Assay, CAL Fluor® 560	**100 reactions**	**AM2030**

For Research Use Only. Not for use in diagnostic procedures. Each system contains sufficient reagents for 100 × 20µl reactions or 200 × 10µl reactions. Includes:

- 1 × 100µl IPC qPCR Inhibition Control, CAL Fluor® 560, 20X
- 1 × 100µl CXR Reference Dye, 30µM

Storage Conditions: Store all components at –30°C to –10°C. Protect components from light at all times. For best results, mix thawed solutions gently to minimize aeration and foaming and keep on ice during use. Do not expose this product to more than 10 freeze-thaw cycles.

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 pipette tips (barrier tips).

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. As a general rule, a concentration of 900nM for PCR primers and 250nM for the hydrolysis probe is a good starting point. Concentrations of PCR primers can range from 200nM to 1µM, while probe concentrations 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. Materials to Be Supplied by the User

- real-time PCR instrument and related consumables (i.e., optical-grade PCR plates and appropriate plate covers)
- GoTaq® Enviro RT-qPCR System (Cat.# AM2010, AM2011)
- GoScript™ Reverse Transcriptase Enzyme (Cat.# A5003)
- Nuclease-Free Water (Cat.# P1193)
- sterile, aerosol-resistant pipette tips (e.g., Barrier Tips, Cat.# A1491)
- nuclease-free pipettors dedicated to pre-amplification work
- primers and probe
- sample containing nucleic acid

Promega

4. Protocol

4.A. Assembling the Reaction Mix with qPCR or RT-qPCR Inhibition Control Assays

The final reaction volume for this protocol is 20µl. The volumes given here can be scaled for larger or smaller reaction volumes.

1. Thaw the IAC or IPC Inhibition Control Assay, GoTaq® Enviro Master Mix, GoScript™ Reverse Transcriptase Enzyme Mix (for RT-qPCR) and Nuclease-Free Water at ambient temperature.
2. Vortex the GoTaq® Enviro Master Mix for 3–5 seconds to mix.
3. 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 amount of extra reagent, the additional volume ensures that you have enough reaction mix for all samples.
4. Prepare the reaction mix (minus the nucleic acid template) by combining the reagents as described in Table 1. Add 1µl of IPC qPCR or IAC RT-qPCR Inhibition Control Assay per 20µl reaction. CXR Reference Dye can be added if the instrument requires it (see your instrument specifications for details). The template is added in Step 6. Vortex briefly to mix.

Table 1. Components and Volumes for qPCR or RT-qPCR Control Assays.

Component	Volume for qPCR Assay	Volume for RT-qPCR Assay	Final Concentration
GoTaq® Enviro Master Mix, 2X	10µl	10µl	1X
GoScript™ Enzyme Mix	—	0.4µl	—
Forward Primer (20X)	1µl	1µl	200nM–1µM
Reverse Primer (20X)	1µl	1µl	200nM–1µM
Hydrolysis Probe (20X)	1µl	1µl	100nM–300nM
CXR Reference Dye[1]	0.02µl/0.33µl	0.02µl/0.33µl	30nM/500nM
IAC RT-qPCR Inhibition Control, CAL Fluor® 560, 20X	—	1µl	—
IPC qPCR Inhibition Control. CAL Fluor® 560, 20X	1µl	—	—
Sample (containing nucleic acid)	2–5µl	2–5µl	≤250ng
Nuclease-Free Water to a final volume of	**20µl**	**20µl**	—

[1]Some instruments may require a reference dye (CXR Reference Dye) at low (30nM) or high (500nM) concentration.

5. Add the appropriate volume of reaction mix to each PCR tube or to each well of an optical-grade PCR plate.
6. Add the nucleic acid template (or Nuclease-Free Water for the no-template control reactions) to the appropriate wells of the reaction plate.

7. Seal the tubes or optical plate. Centrifuge briefly at 300 × *g* to collect the contents at the bottom of the tube or wells. Protect from extended light exposure and elevated temperatures. The samples are now ready for thermal cycling.
8. Analyze the inhibitor control in a CAL Fluor® 560-compatible channel (HEX/JOE/VIC).

5. Appendix

5.A. GoTaq® Enviro Systems Tolerate Environmental Inhibitors

GoTaq® Enviro qPCR and GoTaq® Enviro RT-qPCR Systems are designed to tolerate qPCR and RT-qPCR inhibitors that may be present in environmental samples.

Table 2 shows RT-qPCR reactions performed with the IAC RT-qPCR Inhibition Control Assay (Cat.# AM2040) and either the GoTaq® Enviro RT-qPCR System (Cat.# AM2010) or GoTaq® Probe RT-qPCR System (Cat.# A6120), using 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]

$\Delta C_t > 2$ represents significant inhibition of the reaction

Table 2. GoTaq® Enviro RT-qPCR System Tolerates RT-qPCR 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 RT-qPCR System (ΔC_t)	No C_t	No C_t	7.54	3.75	1.83	1.15	0.1	0

Table 3 shows qPCR reactions performed with the IPC qPCR Inhibition Control Assay (Cat.# AM2030) and either the GoTaq® Enviro qPCR System (Cat.# AM2000) or GoTaq® Probe qPCR Master Mix (Cat.# A6101), using 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 qPCR inhibition.

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

$\Delta C_t > 2$ represents significant inhibition of the reaction

Promega

5.A. GoTaq® Enviro Systems Tolerate Environmental Inhibitors (continued)

Table 3. GoTaq® Enviro qPCR System Tolerates qPCR Inhibitors.

	Humic Acid (ng/reaction)							
Assay	**125**	**62.5**	**31.25**	**15.63**	**7.81**	**3.91**	**1.95**	**0**
GoTaq® Enviro qPCR System (ΔC_t)	0.63	0.2	0	0	0	0	0.02	0
GoTaq® Probe qPCR Master Mix (ΔC_t)	No C_t	No C_t	No C_t	8.84	0.05	0	0	0

5.B. 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.

5.C. Related Products

Product	Size	Cat.#
GoTaq® Enviro qPCR System*	200 reactions	AM2000
	1,000 reactions	AM2001
GoTaq® Enviro RT-qPCR System*	200 reactions	AM2010
	1,000 reactions	AM2011
GoTaq® Probe qPCR Master Mix*	2ml	A6101
	10ml	A6102
GoTaq® Probe 1-Step RT-qPCR System*	2ml	A6120
	12.5ml	A6121
GoTaq® Probe 2-Step RT-qPCR System*	2ml	A6110
Nuclease-Free Water	50ml	P1193
CXR Reference Dye	100µl	C5411

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