Measuring Protocol
qTOWERiris

Optimal Results with qTOWERiris and Genesee Apex Master Mixes

Introduction

Real-time PCR, also known as qPCR, has become an indispensable tool in molecular biology for detecting and quantifying nucleic acids. This technique is essential in several domains, including research and development, the pharmaceutical industry, food and animal feed quality assurance, and forensics. Modern qPCR devices must adhere to increasingly strict quality and performance criteria. In addition to the ability to employ various analytical methods and detect multiple targets simultaneously in multiplex assays, achieving high sensitivity and consistent measurement results is vital. This is primarily ensured by uniform excitation and detection throughout the entire sample block. The qTOWERiris, as highlighted in this Tech Note, meets these rigorous standards by providing optimal homogeneity in measurement results using Genesee Apex qPCR 2x Green Master Mix with different concentrations of ROX.

Your Benefits

- Patented high performance optical system of qTOWERiris Series
- Optimal homogeneous excitation and detection in each of the 96 wells
- High reproducibility and sensitivity

Application

For the SYBR®Green applications, the dye-based master mixes were used in a standard gradient real-time PCR experiment with 8 samples over 12 different temperatures performed in the real-time PCR thermal cycler qTOWERiris. Additionally, the experiment determined different concentrations of the passive reference dye "ROX". The starting material includes human DNA (GAPDH gene as target).

Apex qPCR 2x Green Master Mix	Order number Genesee Scientific
qPCR 2X GREEN Master Mix Without ROX™	42-116PG
qPCR 2X GREEN Master Mix Low ROX™	42-119PG
qPCR 2X GREEN Master Mix High ROX™	42-120PG

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Table 1: Pipetting scheme of 8 replicates human DNA with Apex qPCR 2x Green Master Mix

Volume per reaction:	20 µL
Repeat of determination	8

Device		qTOWERiris		
Master	**Description**	**Stock**	**Final concentration**	**Volume [µL]**
Master Mix	Apex qPCR 2x Green Master Mix	2 x	1 x	80,00 µL
Water				76,48 µL
Template	*Human* DNA	10 ng/µL	0,1 ng/µL	1,6 µL
Primer 1A	GAPDH *fwd*	50 µM	0,5 µM	0,96 µL
Primer 2A	GAPDH *rev*	50 µM	0,5 µM	0,96 µL
		Final Volume per Reaction: (Control)		**160,00 µL**

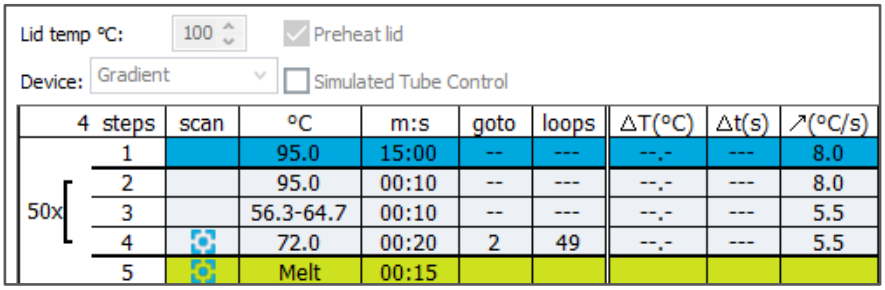

Figure 1: Temperature-time protocol of detection of human DNA with Apex qPCR 2x Green Master Mix

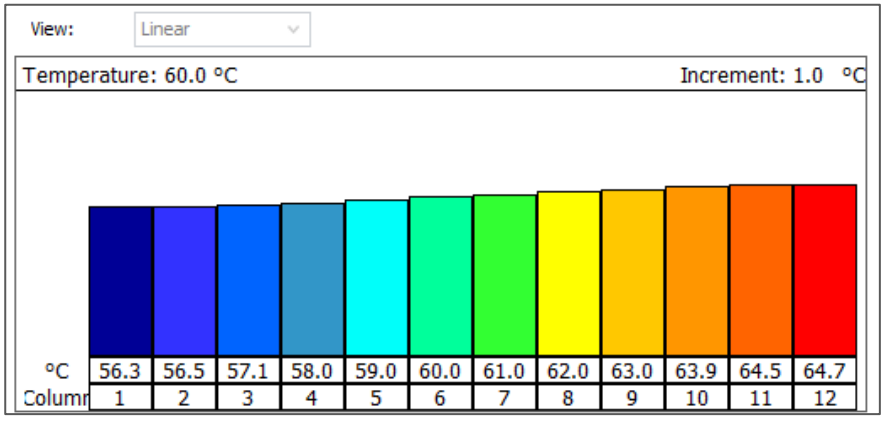

Figure 2: Overview of 8 different annealing temperatures using the Linear Gradient Tool in qTOWERiris

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Results

Apex qPCR 2x Green Master Mix

Temperature Gradient with no ROX™ for passive reference:

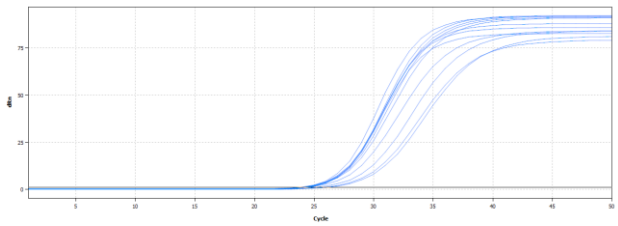

Figure 3: qPCR amplification curves of gradient qPCR experiment over 8 different annealing temperatures (1 row) of human DNA with Apex qPCR 2x Green Master Mix with ***no ROX™*** for passive reference

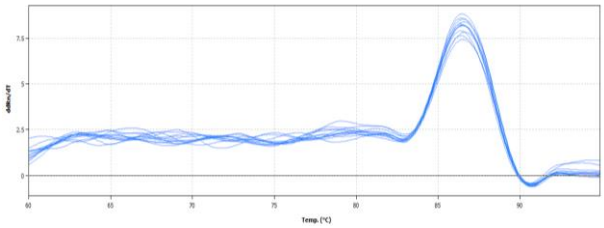

Figure 4: qPCR melting curves of gradient qPCR experiment over 8 different annealing temperatures (1 row) of human DNA with Apex qPCR 2x Green Master Mix with ***no ROX™*** for passive reference

Well	Sample name	Ct
B1	Apex_56.3_NoRox	24.07
B2	Apex_56.5_NoRox	24.35
B3	Apex_57.1_NoRox	24.41
B4	Apex_58.0_NoRox	24.21
B5	Apex_59.0_NoRox	24.26
B6	Apex_60.0_NoRox	24.04
B7	Apex_61.0_NoRox	24.29
B8	Apex_62.0_NoRox	24.22
B9	Apex_63.0_NoRox	24.58
B10	Apex_63.9_NoRox	25.37
B11	Apex_64.5_NoRox	26.03
B12	Apex_64.7_NoRox	26.26

Figure 5: Analysis data of gradient qPCR amplification. (Ct: Cycle threshold)

Well	Sample name	Tm
B1	Apex_56.3_NoRox	86.7
B2	Apex_56.5_NoRox	86.5
B3	Apex_57.1_NoRox	86.4
B4	Apex_58.0_NoRox	86.5
B5	Apex_59.0_NoRox	86.6
B6	Apex_60.0_NoRox	86.5
B7	Apex_61.0_NoRox	86.4
B8	Apex_62.0_NoRox	86.6
B9	Apex_63.0_NoRox	86.4
B10	Apex_63.9_NoRox	86.5
B11	Apex_64.5_NoRox	86.4
B12	Apex_64.7_NoRox	86.5

Figure 6: Analysis data of gradient qPCR melting curve. (Tm: Melting point)

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Temperature Gradient with low ROX™ for passive reference:

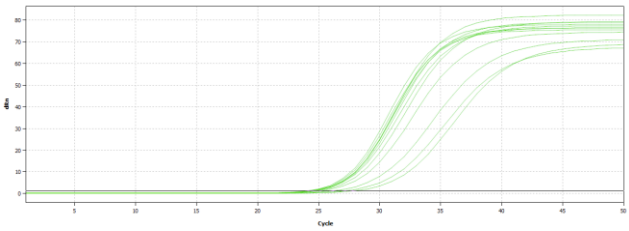

Figure 7: qPCR amplification curves of gradient qPCR experiment over 8 different annealing temperatures (1 row) of human DNA with Apex qPCR 2x Green Master Mix with ***low ROX™*** for passive reference

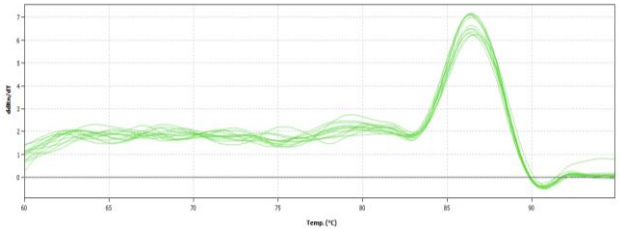

Figure 8: qPCR melting curves of gradient qPCR experiment over 8 different annealing temperatures (1 row) of human DNA with Apex qPCR 2x Green Master Mix with ***low ROX™*** for passive reference

Well		Sample name	Ct
D1		Apex_56.3_LowRox	24.17
D2		Apex_56.5_LowRox	24.23
D3		Apex_57.1_LowRox	24.35
D4		Apex_58.0_LowRox	24.50
D5		Apex_59.0_LowRox	24.37
D6		Apex_60.0_LowRox	24.44
D7		Apex_61.0_LowRox	24.43
D8		Apex_62.0_LowRox	24.70
D9		Apex_63.0_LowRox	25.09
D10		Apex_63.9_LowRox	26.13
D11		Apex_64.5_LowRox	27.08
D12		Apex_64.7_LowRox	27.90

Figure 9: Analysis data of gradient qPCR amplification. (Ct: Cycle threshold)

Well		Sample name	Tm
D1		Apex_56.3_LowRox	86.7
D2		Apex_56.5_LowRox	86.5
D3		Apex_57.1_LowRox	86.5
D4		Apex_58.0_LowRox	86.4
D5		Apex_59.0_LowRox	86.4
D6		Apex_60.0_LowRox	86.4
D7		Apex_61.0_LowRox	86.3
D8		Apex_62.0_LowRox	86.4
D9		Apex_63.0_LowRox	86.4
D10		Apex_63.9_LowRox	86.4
D11		Apex_64.5_LowRox	86.4
D12		Apex_64.7_LowRox	86.5

Figure 10: Analysis data of gradient qPCR melting curve. (Tm: Melting point)

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Temperature Gradient with high ROX™ for passive reference:

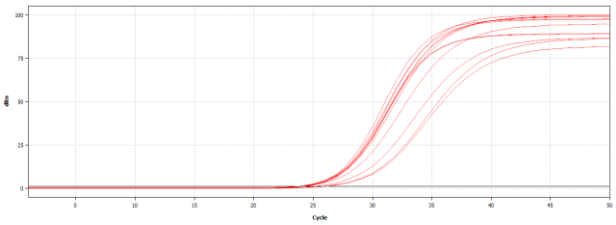

Figure 11: qPCR amplification curves of gradient qPCR experiment over 8 different annealing temperatures (1 row) of human DNA with Apex qPCR 2x Green Master Mix with ***high ROX™*** for passive reference

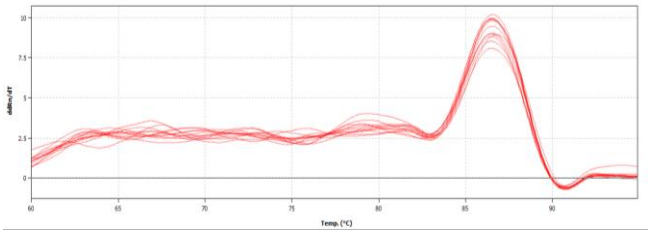

Figure 12: qPCR melting curves of gradient qPCR experiment over 8 different annealing temperatures (1 row) of human DNA with Apex qPCR 2x Green Master Mix with ***high ROX™*** for passive reference

Well	Sample name	Ct
C1	Apex_56.3_HighRox	24.00
C2	Apex_56.5_HighRox	24.21
C3	Apex_57.1_HighRox	24.25
C4	Apex_58.0_HighRox	23.98
C5	Apex_59.0_HighRox	24.01
C6	Apex_60.0_HighRox	23.91
C7	Apex_61.0_HighRox	23.80
C8	Apex_62.0_HighRox	23.89
C9	Apex_63.0_HighRox	24.21
C10	Apex_63.9_HighRox	25.15
C11	Apex_64.5_HighRox	26.08
C12	Apex_64.7_HighRox	25.98

Figure 13: Analysis data of gradient qPCR amplification. (Ct: Cycle threshold)

Well	Sample name	Tm
C1	Apex_56.3_HighRox	86.8
C2	Apex_56.5_HighRox	86.5
C3	Apex_57.1_HighRox	86.5
C4	Apex_58.0_HighRox	86.6
C5	Apex_59.0_HighRox	86.6
C6	Apex_60.0_HighRox	86.5
C7	Apex_61.0_HighRox	86.5
C8	Apex_62.0_HighRox	86.6
C9	Apex_63.0_HighRox	86.5
C10	Apex_63.9_HighRox	86.5
C11	Apex_64.5_HighRox	86.5
C12	Apex_64.7_HighRox	86.6

Figure 14: Analysis data of gradient qPCR melting curve. (Tm: Melting point)

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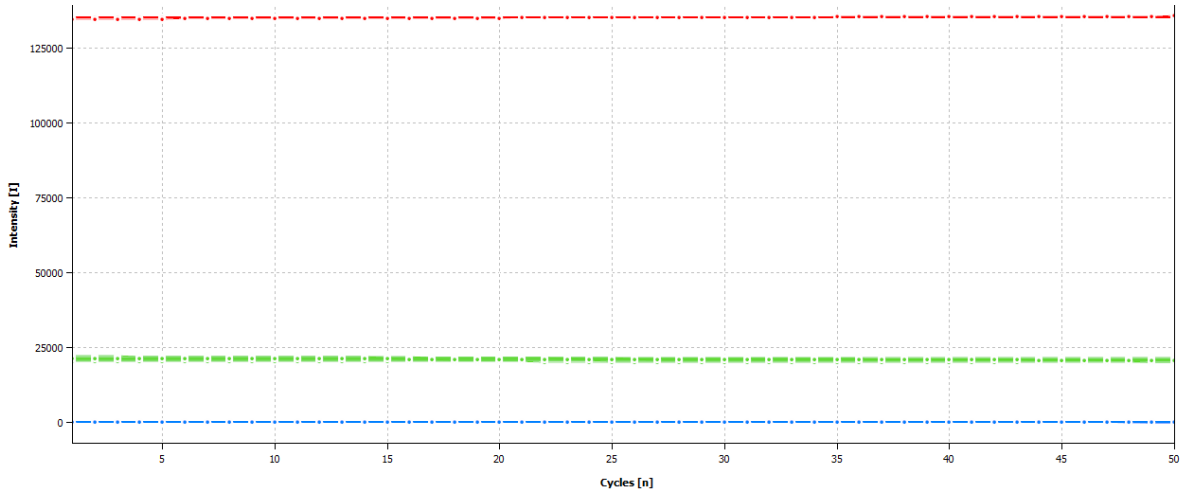

Figure 15: Monitoring data of the intensity data of the passive reference dye of PCR Biosystems qPCRBIO SyGreen Blue Mix (No ROX™ = blue, low ROX™ = green, high ROX™ = red)

Table 2: Average intensities of passive reference dye ROX™

	Intensity
No ROX™	135000
Low ROX™	9300
No ROX™	0

Conclusion

In summary, this test shows that mixes, regardless of the ROX™ concentration, work very well in combination with the real-time PCR thermal cycler qTOWERiris.

The mixes show very stable performance, as despite the temperature gradient of 56.3 – 64.7 degrees in the annealing temperature, only small variations in the Ct value are observed.

The melting points also show almost no variance across the different master mixes.

Therefore, the conclusion is the combination of Genesee Apex qPCR 2x GREEN Master Mix, independent from the ROX™ concentration, together with qTOWERiris is a perfect choice.

Reference: MeasProt_qTOWERiris_ ApexMasterMix_0003_en

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