Measuring Protocol
qTOWERiris

# Optimal results with qTOWERiris and Genesee PCR Biosystem 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 PCR Biosystems qPCRBIO SyGreen Blue 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).

| PCR Biosystems qPCRBIO SyGreen Blue mix | Order number Genesee Scientific |
|---|---|
| PCR Biosystems qPCRBIO SyGreen Blue Mix Separate ROX™ | 17-507 |
| PCR Biosystems qPCRBIO SyGreen Blue Mix Low ROX™ | 17-505 |
| PCR Biosystems qPCRBIO SyGreen Blue Mix High ROX™ | 17-506 |

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Table 1: Pipetting scheme of 8 replicates human DNA with PCR Biosystems qPCRBIO SyGreen Blue Mix

| | |
|---|---|
| **Volume per reaction:** | 20 µL |
| **Repeat of determination** | 8 |

| **Device** | | qTOWERiris | | |
|---|---|---|---|---|
| **Master** | **Description** | **Stock** | **Final concentration** | **Volume [µL]** |
| MasterMix | PCR Biosystems qPCRBIO SyGreen Blue 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** |

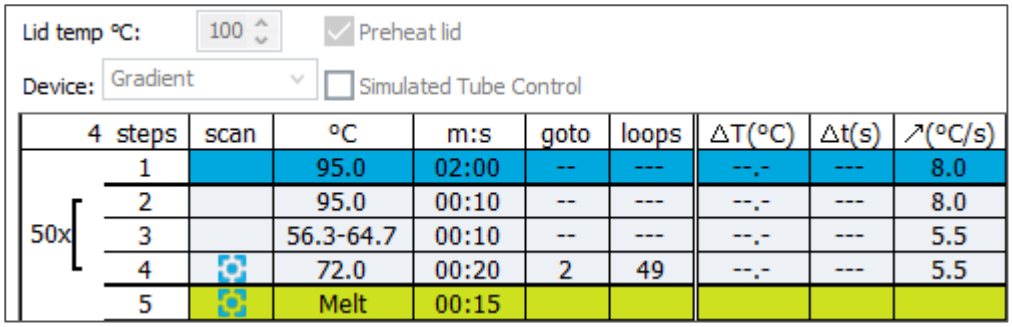


Figure 1: Temperature-time protocol of detection of human DNA with PCR Biosystems qPCRBIO SyGreen Blue mix

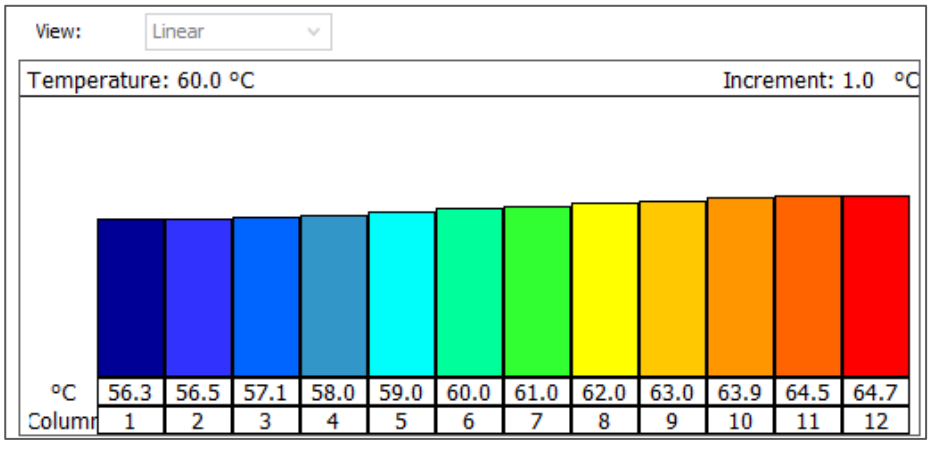


Figure 2: Overview of 8 different annealing temperatures using the Linear Gradient Tool in qTOWERiris

## Results

### PCR Biosystems qPCRBIO SyGreen Blue Mix

### Temperature Gradient with no ROX™ for passive reference:

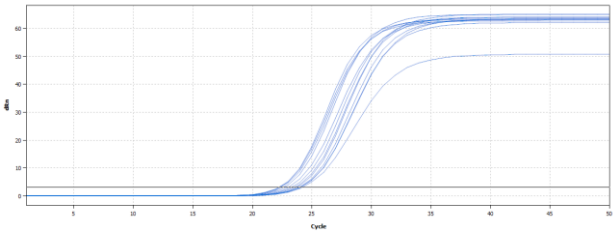

Figure 3: qPCR amplification curves of gradient qPCR experiment over 8 different annealing temperatures (1 row) of human DNA with PCR Biosystems qPCRBIO SyGreen Blue Mix with ***no ROX™*** for passive reference.

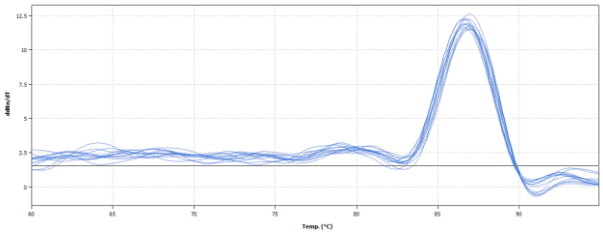

Figure 4: qPCR melting curves of gradient qPCR experiment over 8 different annealing temperatures (1 row) of human DNA with PCR Biosystems qPCRBIO SyGreen Blue Mix with ***no ROX™*** for passive reference.

| Well | Sample name | Ct |
|---|---|---|
| B1 | PCRBiosystems_56.3_NoRox | 22.29 |
| B2 | PCRBiosystems_56.5_NoRox | 22.29 |
| B3 | PCRBiosystems_57.1_NoRox | 22.42 |
| B4 | PCRBiosystems_58.0_NoRox | 22.63 |
| B5 | PCRBiosystems_59.0_NoRox | 23.04 |
| B6 | PCRBiosystems_60.0_NoRox | 23.33 |
| B7 | PCRBiosystems_61.0_NoRox | 23.58 |
| B8 | PCRBiosystems_62.0_NoRox | 23.76 |
| B9 | PCRBiosystems_63.0_NoRox | 24.02 |
| B10 | PCRBiosystems_63.9_NoRox | 24.10 |
| B11 | PCRBiosystems_64.5_NoRox | 24.06 |
| B12 | PCRBiosystems_64.7_NoRox | 24.23 |

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

| Well | Sample name | Tm |
|---|---|---|
| B1 | PCRBiosystems_56.3_NoRox | 86.9 |
| B2 | PCRBiosystems_56.5_NoRox | 86.6 |
| B3 | PCRBiosystems_57.1_NoRox | 86.6 |
| B4 | PCRBiosystems_58.0_NoRox | 86.6 |
| B5 | PCRBiosystems_59.0_NoRox | 86.6 |
| B6 | PCRBiosystems_60.0_NoRox | 86.7 |
| B7 | PCRBiosystems_61.0_NoRox | 86.8 |
| B8 | PCRBiosystems_62.0_NoRox | 86.9 |
| B9 | PCRBiosystems_63.0_NoRox | 87 |
| B10 | PCRBiosystems_63.9_NoRox | 87 |
| B11 | PCRBiosystems_64.5_NoRox | 86.9 |
| B12 | PCRBiosystems_64.7_NoRox | 87 |

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

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## Temperature Gradient with low ROX™ for passive reference:

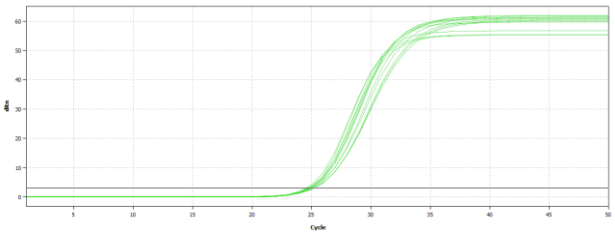

Figure 7: qPCR amplification curves of gradient qPCR experiment over 8 different annealing temperatures (1 row) of human DNA with PCR Biosystems qPCRBIO SyGreen Blue Mix with ***low ROX™*** for passive reference.

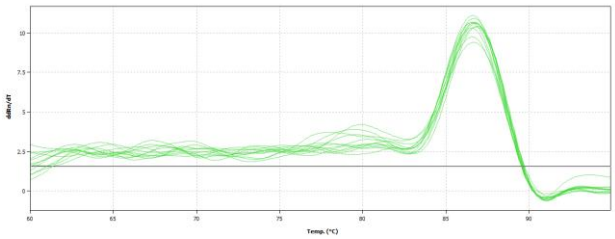

Figure 8: qPCR melting curves of gradient qPCR experiment over 8 different annealing temperatures (1 row) of human DNA with PCR Biosystems qPCRBIO SyGreen Blue Mix with ***low ROX™*** for passive reference.

| Well | | Sample name | Ct |
|---|---|---|---|
| C1 | | PCRBiosystems_56.3_LowRox | 24.57 |
| C2 | | PCRBiosystems_56.5_LowRox | 24.77 |
| C3 | | PCRBiosystems_57.1_LowRox | 24.92 |
| C4 | | PCRBiosystems_58.0_LowRox | 24.90 |
| C5 | | PCRBiosystems_59.0_LowRox | 24.93 |
| C6 | | PCRBiosystems_60.0_LowRox | 25.01 |
| C7 | | PCRBiosystems_61.0_LowRox | 25.02 |
| C8 | | PCRBiosystems_62.0_LowRox | 25.15 |
| C9 | | PCRBiosystems_63.0_LowRox | 25.09 |
| C10 | | PCRBiosystems_63.9_LowRox | 25.31 |
| C11 | | PCRBiosystems_64.5_LowRox | 25.28 |
| C12 | | PCRBiosystems_64.7_LowRox | 25.24 |

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

| Well | | Sample name | Tm |
|---|---|---|---|
| C1 | | PCRBiosystems_56.3_LowRox | 86.7 |
| C2 | | PCRBiosystems_56.5_LowRox | 86.6 |
| C3 | | PCRBiosystems_57.1_LowRox | 86.6 |
| C4 | | PCRBiosystems_58.0_LowRox | 86.5 |
| C5 | | PCRBiosystems_59.0_LowRox | 86.6 |
| C6 | | PCRBiosystems_60.0_LowRox | 86.7 |
| C7 | | PCRBiosystems_61.0_LowRox | 86.7 |
| C8 | | PCRBiosystems_62.0_LowRox | 86.8 |
| C9 | | PCRBiosystems_63.0_LowRox | 86.7 |
| C10 | | PCRBiosystems_63.9_LowRox | 86.7 |
| C11 | | PCRBiosystems_64.5_LowRox | 86.8 |
| C12 | | PCRBiosystems_64.7_LowRox | 86.9 |

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

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## Temperature Gradient with high ROX™ for passive reference:

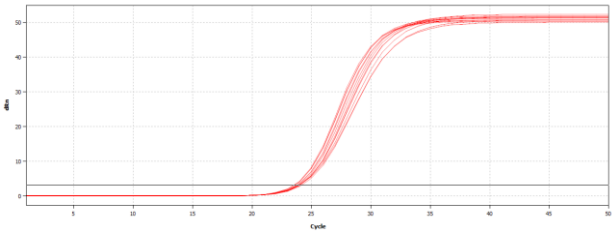

Figure 11: qPCR amplification curves of gradient qPCR experiment over 8 different annealing temperatures (1 row) of human DNA with PCR Biosystems qPCRBIO SyGreen Blue Mix with ***high ROX™*** for passive reference.

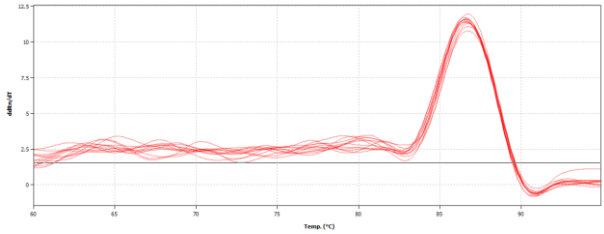

Figure 12: qPCR melting curves of gradient qPCR experiment over 8 different annealing temperatures (1 row) of human DNA with PCR Biosystems qPCRBIO SyGreen Blue Mix with ***high ROX™*** for passive reference.

| Well | | Sample name | Ct |
|---|---|---|---|
| D1 | | PCRBiosystems_56.3_HighRox | 23.56 |
| D2 | | PCRBiosystems_56.5_HighRox | 23.60 |
| D3 | | PCRBiosystems_57.1_HighRox | 23.58 |
| D4 | | PCRBiosystems_58.0_HighRox | 23.79 |
| D5 | | PCRBiosystems_59.0_HighRox | 23.84 |
| D6 | | PCRBiosystems_60.0_HighRox | 23.91 |
| D7 | | PCRBiosystems_61.0_HighRox | 24.08 |
| D8 | | PCRBiosystems_62.0_HighRox | 24.07 |
| D9 | | PCRBiosystems_63.0_HighRox | 24.02 |
| D10 | | PCRBiosystems_63.9_HighRox | 24.05 |
| D11 | | PCRBiosystems_64.5_HighRox | 24.18 |
| D12 | | PCRBiosystems_64.7_HighRox | 24.05 |

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

| Well | | Sample name | Tm |
|---|---|---|---|
| D1 | | PCRBiosystems_56.3_HighRox | 86.8 |
| D2 | | PCRBiosystems_56.5_HighRox | 86.6 |
| D3 | | PCRBiosystems_57.1_HighRox | 86.6 |
| D4 | | PCRBiosystems_58.0_HighRox | 86.5 |
| D5 | | PCRBiosystems_59.0_HighRox | 86.7 |
| D6 | | PCRBiosystems_60.0_HighRox | 86.5 |
| D7 | | PCRBiosystems_61.0_HighRox | 86.6 |
| D8 | | PCRBiosystems_62.0_HighRox | 86.6 |
| D9 | | PCRBiosystems_63.0_HighRox | 86.6 |
| D10 | | PCRBiosystems_63.9_HighRox | 86.7 |
| D11 | | PCRBiosystems_64.5_HighRox | 86.7 |
| D12 | | PCRBiosystems_64.7_HighRox | 86.8 |

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

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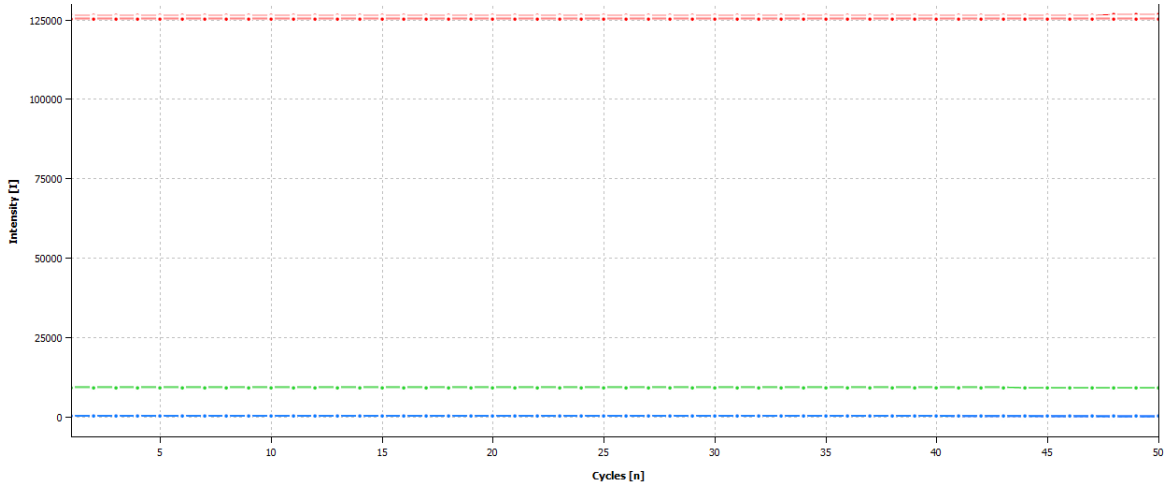


Figure 15: Monitoring data of the intensity data of the passive reference dye of PCR Biosystems qPCRBIO SyGreen Blue Mix (No ROX™ = blue, low ROX™ = green, high ROX™ = red)

Table 2: Average intensities of passive reference dye ROX™

| | Intensity |
|---|---|
| **High ROX™** | 125000 |
| **Low ROX™** | 9000 |
| **No ROX™** | 0 |

## Conclusion

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

The mixes show a 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 can be observed.

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

Therefore, the conclusion is the combination of Genesee PCR Biosystems qPCRBIO SyGreen Blue Mix, independent of the ROX™ concentration, together with qTOWERiris is a perfect choice.

Reference: MeasProt_qTOWERiris_PCRBiosystems_MasterMix_0004_en


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