FastGene® qFYR reaction mix compatibility: Equivalent results with no, low, or high ROX concentrations

Product	FastGene® qFYR Real-Time PCR systems (96-well, 384-well)
Catalog numbers	FG-QPTC01, FG-QPTC02, FG-QPTC03, FG-QPTC04

The following data was provided by the manufacturer: NIPPON Genetics EUROPE GmbH

Summary

This technical note demonstrates that FastGene® qFYR Real-Time PCR Systems deliver equivalent quantification cycle (Cq) values when operated with qPCR master mixes containing no ROX, low ROX, or high ROX. This confirms that FastGene® qFYR, as an open-system platform, can be used confidently with a broad range of commercially available master mixes, including ROX-containing mixes, without altering quantitative results.

Introduction

FastGene® qFYR Systems are specified as no ROX real-time PCR platforms. In practice, many laboratories routinely use master mixes that contain ROX passive reference dye (often labeled as low ROX or high ROX mixes). This can raise a practical question: Will ROX-containing mixes affect quantification on the qFYR system? This technical note demonstrates that the qFYR produces equivalent Cq values with no ROX, low ROX, and high ROX master mixes.

ROX is a passive reference fluorophore. Unlike reporter dyes, ROX does not indicate target amplification. Instead, it provides a reference signal that some qPCR platforms use to normalize fluorescence measurements

and reduce non-PCR-related variability. Such variability can arise from differences in optical illumination or detection across wells, gradual signal drift over time, and artifacts such as bubbles or sample movement that alter the measured fluorescence intensity. ROX normalization is therefore primarily an instrument- and workflow-dependent process: it is most beneficial on systems where optical non-uniformity or drift influences fluorescence readout, and it is less critical on systems designed to minimize these effects at the acquisition level.

FastGene® qFYR Systems use a well-to-well scanning optical shuttle that measures each well individually in every cycle. Because the scanning head operates very close to the plate, the optical path is short, which reduces signal loss and results in virtually no inter-well cross-talk. The scanning head integrates multiple colour channels for excitation/detection so that each well is acquired channel-by-channel during the plate scan. In many other qPCR designs, fluorescence is recorded through a shared optical path and separated into channels using filters/beam-splitting and color compensation depending on the instrument architecture. This per-well, per-cycle scanning principle and channel-specific acquisition contribute to highly consistent fluorescence readout on qFYR and therefore eliminate the need for ROX-based passive reference normalization. While ROX is not required, qFYR remains compatible with ROX-containing master mixes, and the data in this note demonstrates that ROX presence does not affect Cq values.

Materials & methods

A DNA standard curve was measured on the qFYR using the same assay and cycling conditions under three ROX conditions: no ROX, low ROX (50 nM), and high ROX (500 nM).

Instrument:

- FastGene® qFYR Real-Time PCR System (FG-QPCT01)

Reagents and consumables:

- qPCR master mix: FastGene® IC Green Mix (FG-LS4001)
- Plate: FastGene® White 96-well plate (FG-210250)
- Seal: FastGene® PCR sealing foil (FG-93AC)
- Primers: Standard kit forward and reverse primers
- ROX additive: 50 µM ROX solution

Standard curve preparation:

- Number of points: 5-point standard curve
- Dilution series: 10-fold serial dilutions (1.2×10^6 to 1.2×10^2 copies)
- Triplicates

Table 1: Master mix preparation volumes

Cycles	Temperature	Time	Note
1	95 °C	1 minute	Polymerase activation
40	95 °C	15 seconds	Denaturation
	63 °C	45 seconds	Anneal/extension

Results

Across all tested ROX conditions, qFYR produced equivalent Cq values for no ROX, low ROX (50 nM), and high ROX (500 nM) (Table 2, Figure 1). The standard curve performance metrics (slope, linearity, efficiency, and regression error) were also highly similar between conditions, indicating that ROX concentration did not affect quantitative performance on qFYR (Table 2). A modest increase in overall fluorescence may be observed in reactions containing high ROX (Figure 1, green curves). This is expected because ROX is an additional fluorophore present in the reaction mixture and can contribute to the overall fluorescence signal. Importantly, this change in signal appearance does not translate into a Cq shift.

Table 2: Mean Cq values for the five-point (Standard 1-5), 10-fold dilution series (1.2×10^6 to 1.2×10^2 copies per reaction), measured in triplicate under no ROX, low ROX, and high ROX conditions.

	Standard 1 [Cq]	Standard 2 [Cq]	Standard 3 [Cq]	Standard 4 [Cq]	Standard 5 [Cq]
No ROX	9,88	13,08	16,30	19,69	23,03
Low ROX	9,60	13,06	16,20	19,58	23,07
High ROX	9,77	13,25	16,62	19,81	23,15

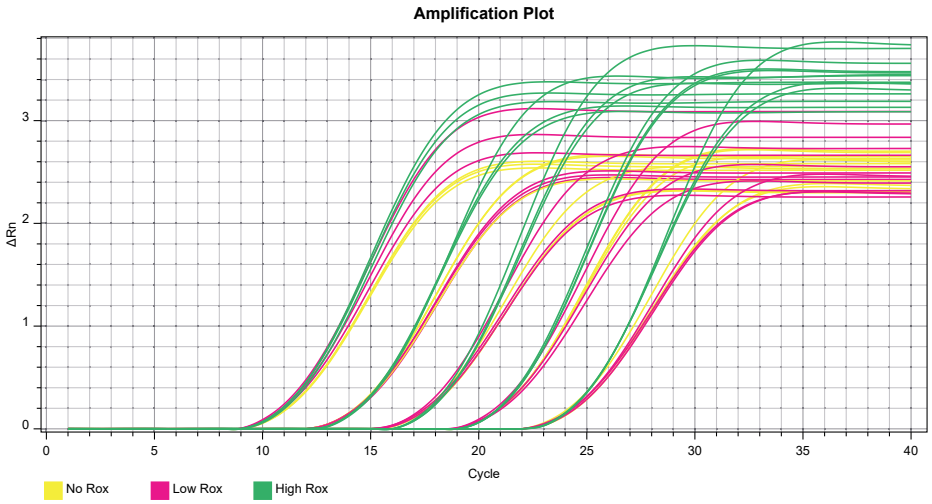

Figure 1: Overlay of amplification standard curves for no ROX (yellow), low ROX (50 nM, pink), and high ROX (500 nM, green) conditions. The curves closely overlap in the exponential amplification phase, illustrating equivalent quantification across ROX concentrations on the qFYR.

Table 3: Standard curve quality metrics for each ROX condition, including slope, R^2, efficiency (%), and standard regression error.

	Slope	R^2	Efficiency [%]	Standard regression error (Cq)
No ROX	-3,29	0,9997	101,30	0,017
Low ROX	-3,35	0,9996	99,05	0,019
High ROX	-3,32	0,9996	100,08	0,018

Conclusion

This technical note confirms that the qFYR systems are fully compatible with qPCR master mixes containing no ROX, low ROX, or high ROX. Under standardized data analysis conditions, Cq values and standard curve performance metrics (slope, R^2, efficiency, and regression error) were equivalent across all ROX conditions. This demonstrates that ROX presence does not affect quantitative results on the qFYR.

ROX is commonly used on qPCR platforms as a passive reference dye to reduce non-PCR fluorescence variability, mostly associated with optical non-uniformity. qFYR's well-to-well scanning acquisition, with the optical unit measuring each well individually in every cycle and acquiring data in all channels, is designed to deliver consistent fluorescence readout without requiring ROX-based normalization.

However, users should not be unsettled by the "no ROX instrument" designation. qFYR is an open system that enables flexible reagent selection, including premixed ROX-containing master mixes. It maintains consistent quantification and simplies workflows by removing the need to add or manage a passive reference dye.

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