Product

FastGene® 2x IC Green Blue Mix Demo Kit

Catalog #

LS42s (20 rxns)

1. Description

The FastGene® 2x IC Green Blue Mix Demo Kit is specifically designed to support reliable, accurate, and reproducible qPCR workflows using the FastGene® qFYR (Plus) Real-Time PCR System.
This kit is ideal for demonstration purposes, particularly by distributors or end-users looking to validate the performance and ease of use of the qFYR (Plus) system. It enables a robust and straightforward protocol, making it perfect for showcasing the system‘s accuracy, sensitivity, and reliability:

- Includes 5 high-quality DNA standards and Demo Kit primers (forward and reverse)
- IC Green® dye-based chemistry
- Blue Mix formulation improves pipetting visibility and ease of use
- Antibody-mediated hot start polymerase ensures specificity and performance

2. Content

Table 1: Content of the FastGene® 2x IC Green Blue Mix Demo Kit

Component	Description	Volume
2x FastGene IC Green® Blue Mix	Ready to use mix with reaction buffer, polymerase enzyme and blue dye to improve pipetting visibility and ease of use. No Rox.	1 x 200 µL
DNA Standards 1-5	DNA standards - serially diluted by a factor of 10, corresponding to 1.2 x 10^6 to 1.2 x 10^2 copies per reaction.	20 µL each
10x Demo Kit Primers	Contains forward and reverse primers	1 x 40 µL

3. Demo Kit reaction setup

3.1 Reaction volume

This kit is designed for one Standard Curve demonstration experiment measured in triplicates, each with a total PCR reaction volume of 20 µl. This includes a total of 16 reactions:

- 5 DNA standards, each in triplicate (15 reactions)
- 1 Negative control (1 reaction)

Optional: You may run the negative control in triplicate, increasing the total to 18 reactions. Please note that the kit contains a maximum of 20 reactions.

3.2 Master mix preparation

- Prepare a master mix (Table 2) for 17 reactions (or 19, if triplicate negative controls are used) to ensure sufficient master mix volume and minimize pipetting errors.
- Before preparing the master mix, briefly vortex and spin down all components.

Table 2: Master mix preparation volumes

Component	**Master Mix Volume for 17 reactions** **(1x negative control)**	**Master Mix Volume for 19 reactions** **(Triplicate negative control)**
2x FastGene IC Green® Blue Mix	170 µl	190 µl
10x Demo Kit primers	34 µl	38 µl
PCR grade dH_2O*	68 µl	76 µl
Total Master Mix Volume	*272 µl*	*304 µl*

* not included in the Kit

3.3 PCR plate preparation

1. Dispense 16 µl of the prepared master mix into each well of a 0.1 ml PCR plate or 8-well strip (Please refer to section 4.1 for the possible pipetting schemes and plate layout).
2. Add 4 µl of each DNA standard into the respective wells (triplicates for standards 1–5).
3. For the negative control(s), add 4 µl of PCR-grade dH_2O in place of DNA standard. Note: PCR-grade dH_2O is not included in the kit.

3.4 Important Notes

1. Always use PCR-grade dH_2O (not tap or distilled water) for reliable results.
2. Include a negative control to check for contamination.
3. Mix gently and briefly spin down the plate before placing it in the thermal cycler.

4. qFYR Software setup

Pre-configured FastGene® qFYR software Demo Kit templates are available and ready for immediate download. The template should match the device being used (qFYR or qFYR PLUS). The templates include the optimized PCR cycling program and plate setup, eliminating the need for manual configuration.
For manual configuration refer to section 4.1.
You can download a pre-configured setup file from the demo kit webpage:

LS42s Webpage Link:
https://www.nippongenetics.eu/en/products/pcr-reagents-and-enzymes/qpcr/ic-green-qpcr/fastgene-2x-ic-green-blue-mix-demo-kit/

4.1 PCR cycling program and plate setup

1. Program the FastGene® qFYR software according to the thermal profile in table 3:

Table 3: 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

2. Configure the plate setup in the software according to your pipetting scheme. Figure 1 shows a possible pipetting scheme

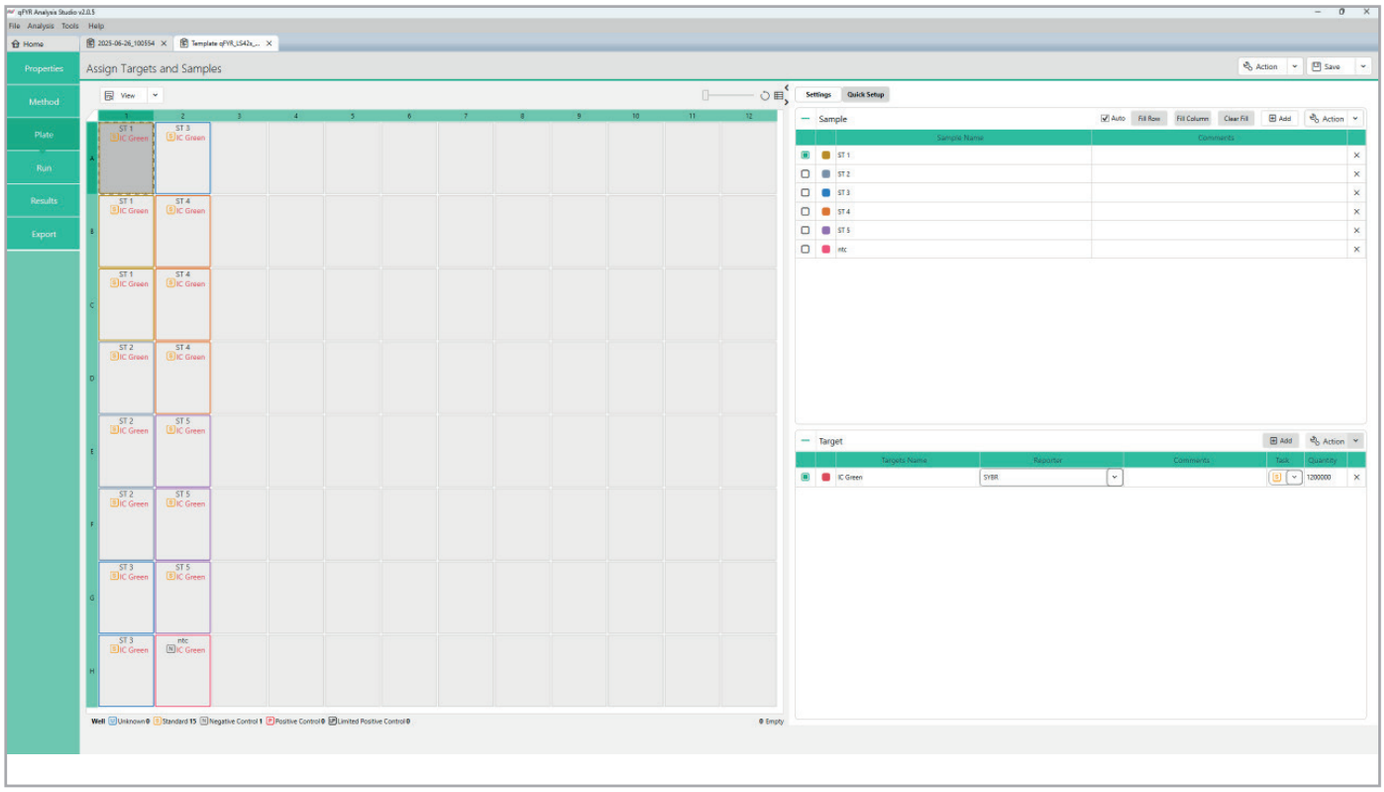

Figure 1: Demo Kit Standard Curve experimet pipetting scheme and plate setup in the qFYR software.

5. Data analysis

The demo kit delivers an excellent standard curve, with high amplification efficiency and a strong correlation coefficient (R^2), making it ideal for performance validation (Figure 2 and figure 3).

Please note: A late amplification in the negative control can occur due to primer-dimers or artifacts, but if the Cq is higher than all standards, it can be considered negative.

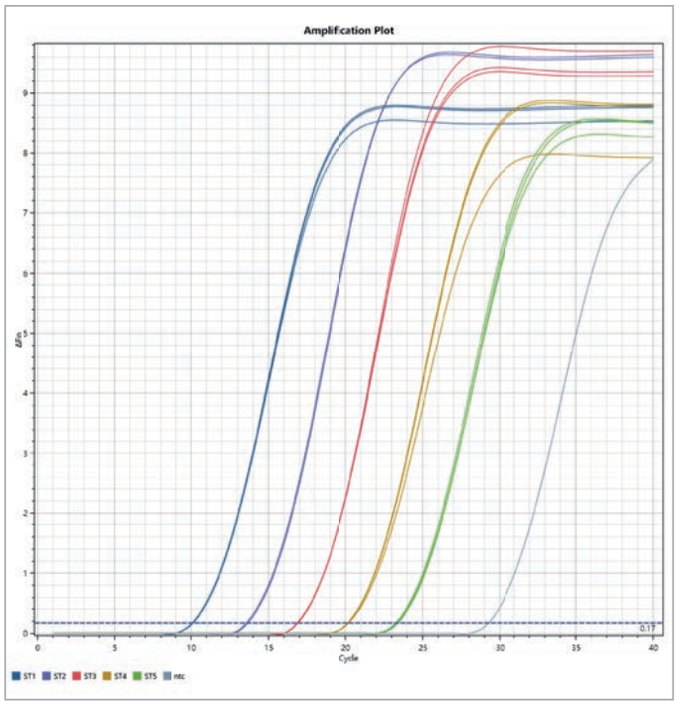

Figure 1: Amplification curves for the 5 DNA standards included in the FastGene® 2x IC Green Blue Mix Demo Kit, demonstrating consistent performance across a wide dynamic range. The clear separation between standard concentrations highlights the assay's sensitivity and reproducibility.

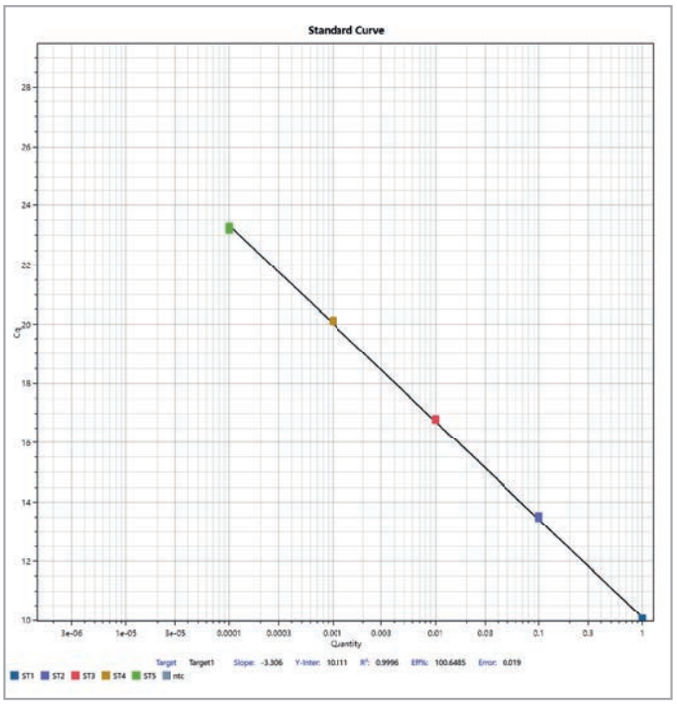

Figure 1: Standard curve generated from the demo kit showing excellent linearity (R^2 = 0.9996) and high efficiency (100.65%). This confirms the robustness and accuracy of the FastGene® qFYR System when using the demonstration kit.

6. Shipping and Storage

On arrival the kit should be stored between -30 and -20 °C. Avoid prolonged exposure to light. If stored correctly, the kit will retain full activity until the indicated expiry date. The kit can be stored at 4 °C for 1 month.

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