February 2024

Quick-Start Protocol

Investigator® Quantiplex® Pro FLX for Applied Biosystems® 7500 and QuantStudio™ 5 Real-Time PCR Systems

The Investigator Quantiplex Pro FLX Kit (cat. no. 387516) is shipped at 2–8°C. The Quantiplex Pro FLX plates can be stored at room temperature (15–25°C), protected from light in the aluminum bags. Partially used plates can be put back into their aluminum bag, including the desiccant, and stored for up to 4 weeks until being used up completely. The QuantiTect Nucleic Acid Dilution Buffer and the Control DNA M1 should be stored at 2–8°C. Under these conditions, the components are stable until the expiration date indicated on the kit.

Further information

- *Investigator Quantiplex Pro FLX Handbook*: **www.qiagen.com/HB-3538**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Do not open plates in advance. Remove seals just before starting setup.
- Before removing the seals from the plate, make sure lyophilized cakes do not stick to the seals. Tap the plates on the bench to bring all cakes down.
- Loosened cakes, spots at the tube wall, or cakes with slightly varying appearance do not have an impact on the performance of the assay.

- Avoid high humidity in the laboratory. We do not recommend to open plates in an environment with over 60% humidity.
- We strongly recommend to always start with the addition of the higher volume during setup. If using >9 µL sample input, add sample first to dissolve the lyophilized cake, and then fill up to the final volume with water (e.g., PCR Water, cat. no. 17000-10). Do pipette above the cake surface.
- Set up all reaction mixtures in an area separate from that used for DNA isolation and PCR product analysis (post-PCR).
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- Dilutions of DNA quantification standards in QuantiTect Nucleic Acid Dilution Buffer can be stored between 2–8°C for at least 1 week.
- Reaction setup can be done at room temperature (15–25°C).
- Always use the cycling conditions specified in the protocol. The cycling conditions have been optimized for this assay.
- Optimal analysis settings are a prerequisite for accurate quantification data. Always readjust the analysis settings (i.e., threshold values) for analysis of every target in every run.
- Download the template files from the **Resources** tab of the product page.

Procedure

1. Prepare fresh serial dilutions of the Male Control DNA M1. Vortex for at least 5 s, and centrifuge each dilution briefly before removing an aliquot for the next dilution. Use a new pipette tip for each dilution. Make sure not to introduce cross-contamination.

Table 1. Serial dilution of Control DNA M1

Serial dilution of Control DNA M1 (ng/µL)	Control DNA (µL)	QuantiTect Nucleic Acid Dilution Buffer (µL)
50	Undiluted DNA	–
1.8519	5	130
0.0686	5	130
0.0025	5	130

2. Thaw samples for quantification if they were stored frozen.
3. When not using the entire plate, break the desired number of 8-tube strips from the plate.

   Note: The unused part of the sealed PCR plate should be stored in the aluminum bag with desiccant and used up within 4 weeks.
4. Peel the perforated seals from the plate, or groups of 8-tube strips. When using the entire plate, seals can be removed with the help of 1/3 of an adhesive foil. Start setting up reactions immediately.
5. Add 18 µL water or Nucleic Acid Dilution Buffer to the NTC (No Template Control) wells.
6. Add 16 µL water or Nucleic Acid Dilution Buffer and 2 µL control DNA M1 dilutions to the individual wells of the standard row. It is required to run duplicates of the control DNA dilutions for each assay and on each reaction plate.
7. Add samples to reaction wells (variable input volumes from 1–18 µL). If using less than 9 µL sample input, start the setup with adding the volume of water required to give a final volume of 18 µL added to each cake.
8. Add water where still needed to all samples or controls to result in a total liquid volume of 18 µL. Never run samples with volume less than 18 µL.
9. Seal the plate, or the 8-tube strips with the qPCR adhesive plate foil (cat. no. 386055 or 209003) and vortex for 5 s.
10. Centrifuge briefly.

Table 2. Reaction setup

Component	1 Reaction
Quantiplex Pro FLX Reaction Mix and Primer Mix	Lyophilized cake
Sample	Variable 1–18 µL
Water	Fill up to 18 µL
Total reaction volume	**20 µL***

* Lyophilized cake + 18 µL input volume will yield a final PCR reaction volume of 20 µL.

Note: If your sample input volume is not 2 µL, a correction factor needs to be applied to your sample concentration. You can also use the QIAGEN Quantification Assay Data Handling Tool for automated concentration correction calculation.

11. Continue with the amplification and analysis as described for the corresponding thermal cycler.

    Note: Always use a compression pad if using the entire plate. If using 8-tube strips, please follow the PCR cycler manufacturer's recommendation for the use of strips.

Table 3. Cycling protocol

Step	Temperature (°C)	Time	No. of cycles	Remarks
Initial PCR activation step	98	3 min	–	PCR requires an initial incubation at 98°C to activate the DNA polymerase
Denaturation	98	5 s	40	–
Combined annealing/extension	65	35 s		Perform fluorescence data collection

Document Revision History

Date	Changes
02/2024	Initial release

Scan QR code for handbook.