

Investigator[®] Quantiplex[®] Pro Kit Using the Applied Biosystems[®] 7500/7500 Fast Real-Time PCR System or 7500 Real-Time PCR System for Human Identification

Kit reagents should be stored immediately upon receipt at -30 to -15°C in a constant-temperature freezer. After first use, store the kit components at 2 – 8°C ; avoid freezing the kit components. The QuantiTect[®] Nucleic Acid Dilution Buffer may also be stored at -30°C to -15°C , if desired. Primer Mix must be stored protected from the light. DNA samples should be stored separately from PCR reagents. Under these conditions, the components are stable until the expiration date indicated on the kit.

Further information

- *Investigator Quantiplex Pro Handbook*: www.qiagen.com/HB-2335
- Safety Data Sheets: www.qiagen.com/safety
- Technical assistance: support.qiagen.com

Notes before starting

- 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.
- Always use the cycling conditions specified in the protocol. The cycling conditions have been optimized for this assay.

- Always use the template volume specified in the protocol. The reaction is optimized for use with 2 µl template DNA. Do not use more or less than 2 µl per 20 µl reaction.
- Dilutions of DNA quantification standards in QuantiTect Nucleic Acid Dilution Buffer can be stored at 4°C for at least 1 week.
- Optimal analysis settings are a prerequisite for accurate quantification data. Always readjust the analysis settings (i.e., threshold values and baseline settings) for analysis of every target in every run.
- Download the template files from the product resources page at:
www.qiagen.com/QPpro-template-files

1. Thaw kit components, if required. Mix all kit components before use.
2. Prepare fresh serial dilutions of the Male Control DNA M1 according to Table 1. Vortex for at least 5s and centrifuge each dilution briefly before removing an aliquot for the next dilution. Use a new pipet tip for each dilution.

Table 1. Serial dilutions of Male Control DNA M1

Serial dilution of Control DNA M1	Control DNA M1	QuantiTect Nucleic Acid Dilution Buffer
50 ng/µl	Undiluted DNA	–
1.8519 ng/µl	5 µl	130 µl
0.0686 ng/µl	5 µl	130 µl
0.0025 ng/µl	5 µl	130 µl
NTC*	–	6 µl

* NTC: No-template control.

3. Prepare a master mix according to Table 2.

Note: Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. This should include positive and negative control reactions.

Table 2. Reaction mix setup

Component	Volume per 20 µl reaction
Quantiplex Pro Reaction Mix	9 µl
Quantiplex Pro Primer Mix	9 µl
Total volume of master mix	18 µl

- Mix the master mix thoroughly and dispense 18 µl into each well.
- Add 2 µl QuantiTect Nucleic Acid Dilution Buffer to the NTC tubes.
- Add 2 µl control DNA dilutions or 2 µl unknown sample DNA to the individual wells and mix thoroughly. Close the plate. Centrifuge briefly.
- Open the Applied Biosystems 7500 Software v 2.0.6, click **File**, then open the template file or if you are using the HID Real-Time PCR Analysis Software v1.1/ v1.2, open the software in **Custom Assays** mode, then select **New Experiment From Template**.
- Select the **Template** file.
- Confirm that the cycling conditions preset on the Applied Biosystems 7500/7500 Fast Real-Time PCR System (Standard Mode) or 7500 Real-Time PCR System for Human Identification are the same as outlined in Table 3.

Table 3. Cycling protocol using an Applied Biosystems 7500/7500 Fast Real-Time PCR System or 7500 Real-Time PCR System for Human Identification

Step	Temp	Time	Number of cycles	Additional comments
Initial PCR activation step	95°C	3 min	–	PCR requires an initial incubation at 95°C to activate the DNA polymerase
Two-step cycling:			40	
Denaturation	95°C	5 s		
Combined annealing/ extension	60°C	35 s		Perform fluorescence data collection

10. Click **Start Run**.

Note: Detailed protocols and instructions for data analysis are available in the *Investigator Quantiplex Pro Handbook*.



Scan QR code for handbook.

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