Quick-Start Protocol September 2017

# Investigator® Quantiplex® Pro RGQ Kit Using the Rotor Gene® Q

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 RGQ Handbook*: **www.qiagen.com/HB-2492**
- 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-rgq-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 5 s, 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 RGQ Reaction Mix | 9 µl |
| Quantiplex Pro RGQ Primer Mix | 9 µl |
| Total volume of master mix | 18 µl |

4. Mix the master mix thoroughly and dispense 18 µl into each tube.
5. Add 2 µl QuantiTect Nucleic Acid Dilution Buffer to the NTC tubes.
6. Add 2 µl control DNA dilutions or 2 µl unknown sample DNA to the individual tubes and mix thoroughly.
7. Close the PCR tubes. Place them in the appropriate rotor in the Rotor-Gene Q cycler, and attach the locking ring.
8. If you are using tubes, empty positions in the rotor should be filled with empty PCR tubes.
9. Open the Q-Rex Software 1.0, then select and open the appropriate template file.
10. Confirm that the cycling conditions preset in the template file are the same as outlined in Table 3.

**Table 3. Cycling conditions for the Rotor-Gene Q**

| Step | Temperature | 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 | 10 s | | Perform fluorescence data collection |

11. Click "Start Run".

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

Scan QR code for handbook.