Quick-Start Protocol June 2018

Investigator® Quantiplex® HYres Kit on the Rotor-Gene® Q

Store the kit reagents at 2–8°C. Avoid freezing these kit components. The QuantiTect® Nucleic Acid Dilution Buffer may also be stored at –30°C to –15°C, if desired. Avoid repeated freezing and thawing. Primer Mix IC YQ must be stored protected from 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 HYres Handbook*: **www.qiagen.com/HB-1155**
- 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 risks.
- Always use the cycling conditions specified in the protocol. The cycling is 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.
- We recommend using a 72-well rotor.
- 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., baseline settings and threshold values) for analysis of every reporter dye channel in every run.
- Download the template files for the *Investigator Quantiplex HYres Kit*.

Procedure

1. 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

Standard	Serial dilution of Control DNA (ng/µl)	Control DNA (ng/µl)	QuantiTect Nucleic Acid Dilution Buffer (µl)
–	50 (stock conc.)	–	–
Standard -1	20	20 (from stock)	30
Standard -2	5	10	30
Standard -3	1.25	10	30
Standard -4	0.3125	10	30
Standard -5	0.078125	10	30
Standard -6	0.01953125	10	30
Standard -7	0.0048828125	10	30

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 setup

Component	Volume per 20 µl reaction
Reaction Mix YQ	9 µl
Primer Mix IC YQ	9 µl
Total volume of master mix	**18 µl**

4. Mix the master mix thoroughly and dispense 18 µl into Rotor-Gene Q Strip Tubes.
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 PCR tubes.
7. Close the PCR tubes. Place the tubes in the 72-well rotor in the Rotor-Gene Q cycler and attach the locking ring.
8. Open the Rotor-Gene Software. In the Quick-Start Wizard, select **Open A Template In Another Folder…** and load the Investigator Quantiplex HYres template file.
9. Confirm that the locking ring is attached and click **Next**.
10. Confirm that the cycling conditions pre-set on the Rotor-Gene Q are the same as outlined in Table 3.

Table 3. Cycling protocol on the Rotor-Gene Q

Step	Time	Temperature	Number of cycles	Comment
Initial activation step	3 min	95°C	–	PCR requires an initial incubation at 95°C to activate the DNA polymerase.
Two-step cycling:			**40**	
Denaturation	5 s	95°C		
Combined annealing/extension	10 s	60°C		Perform fluorescence data collection using the green, red, and yellow channels with auto-gain optimization.

11. Click **Next** and **Start Run**.

Note: Detailed protocols for other real-time cyclers, as well as instructions for data analysis, are available in the *Investigator Quantiplex HYres Handbook*, which can be found at **www.qiagen.com/HB-1155**.

Scan QR code for handbook.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual.

Document Revision History	
R5 06/2018	Replacement of Control DNA1 Z1 with Male Control DNA M1. Updated the table with DNA concentrations and updated cycling protocol for Rotor-Gene Q.
R4 03/2016	Layout updates.
R3 11/2014	Reaction Mix FQ changed to Reaction Mix YQ (Table 2). Layout updates.