

Rotor-Gene® Type-it® HRM® Discovery Kit

The Rotor-Gene Type-it HRM Discovery Kit (cat. no. 206541), including reagents, should be stored immediately upon receipt at -20°C in a constant-temperature freezer. The 2x HRM PCR Master Mix can also be stored at $2-8^{\circ}\text{C}$ for up to 2 months.

For more information, please refer to the *Rotor-Gene Type-it HRM Discovery Handbook*, which can be found at www.qiagen.com/handbooks.

For technical assistance, please call toll-free 00800-22-44-6000, or find regional phone numbers at www.qiagen.com/contact.

Notes before starting

- The Rotor-Gene Type-it HRM Discovery Kit demonstrates HRM analysis on the Rotor-Gene Q. The kit provides 2 HRM genotyping assays — a class IV SNP transversion mutation assay and a deletion assay.
- All necessary reagents are supplied. Please refer to the *Rotor-Gene Type-it HRM Discovery Handbook* for detailed information on kit content, as well as additional equipment required for use with this kit.
- Always follow the pipetting schemes specified in the *Rotor-Gene Type-it HRM Discovery Handbook*.
- For automated reaction setup using the QIAgility®, please refer to the *Rotor-Gene Type-it HRM Discovery Handbook* and the *QIAgility User Manual*.

Table 1. Protocols for use with the Rotor-Gene Type-it HRM Discovery Kit

	Protocol
Protocol 1:	Manual reaction setup
Protocol 2:	PCR and HRM analysis on the Rotor-Gene Q

Protocol 1: Manual reaction setup

1. Thaw the 2x HRM PCR Master Mix, standards, unknown samples, and RNase-free water. Mix all solutions well before use to avoid localized concentrations of salt.
2. Prepare a reaction mix according to Table 2 (class IV SNP assay) or Table 3 (deletion assay).

Table 2. Manual reaction setup when analyzing the class IV SNP assay

Component	Volume/25 μ l reaction	Volume/520 μ l reaction*	Final concentration
2x HRM PCR Master Mix	12.5 μ l	325 μ l	1x
Class IV SNP Primer Mix	1.75 μ l	45.5 μ l	0.7 μ M
RNase-free water	5.75 μ l	149.5 μ l	–
DNA (unknown samples or standards; added at step 4)	5 μ l	–	1 x 10 ⁵ copies

* A 520 μ l reaction mix is equivalent to 26 reactions comprising 25 μ l each.

Table 3. Manual reaction setup when analyzing the deletion assay

Component	Volume/25 μ l reaction	Volume/680 μ l reaction [†]	Final concentration
2x HRM PCR Master Mix	12.5 μ l	425 μ l	1x
Deletion Primer Mix	1.75 μ l	59.5 μ l	0.7 μ M
RNase-free water	5.75 μ l	195.5 μ l	–
DNA (unknown samples or standards; added at step 4)	5 μ l	–	1 x 10 ⁵ copies

[†] A 680 μ l reaction mix is equivalent to 34 reactions comprising 25 μ l each.

3. Mix the reaction thoroughly and dispense 20 μ l volumes into PCR tubes. Use six 0.1 ml strip tubes and caps if analyzing the SNP and eight 0.1 strip tubes and caps if analyzing the deletion.

Note: Each strip contains 4 tubes.

4. Add 5 μ l DNA (standards or unknown samples) to each PCR tube. For the No Template Control (NTC), add RNase-free water instead. Please refer to recommended pipetting schemes specified in Table 2 (class IV SNP) or Table 4 (deletion) of the *Rotor-Gene Type-it HRM Discovery Handbook*.
5. After reaction setup, proceed to 'Protocol 2: PCR and HRM analysis on the Rotor-Gene Q'.

Note: A protocol for automated reaction setup using the QIAgility is provided in the *Rotor-Gene Type-it HRM Discovery Handbook*.

Protocol 2: PCR and HRM analysis on the Rotor-Gene Q

Please refer to the *Rotor-Gene Q User Manual* before using the Rotor-Gene Q.

1. Close the PCR tubes containing the reactions. Place the tubes in the 72-well rotor in the Rotor-Gene Q and attach the locking ring.

Note: Empty positions in the 72-well rotor should be filled with empty PCR tubes.

2. Open the ready-made protocol file "Rotor-Gene Type-it HRM Discovery Kit.ret" by double-clicking on it. Alternatively, open the Rotor-Gene Q software and select the "HRM" folder in the advanced tab of the wizard. Choose the protocol folder entitled "HRM with Pre-Amplification" and click on "New".

Note: The ready-made protocol file can be downloaded from www.qiagen.com.

3. Select "72-Well Rotor" and confirm that the locking ring has been attached by checking the box. Click "Next" to continue.
4. Ensure that the reaction volume is set to 25 μ l. Click "Next" to continue.

Quick-Start Protocol

- Click on “Edit Profile” and program the Rotor-Gene Q according to the program outlined in Table 4. Refer to the *Rotor-Gene Type-it HRM Discovery Handbook* for further information.

Note: Data acquisition should be performed during the combined annealing/extension step.

- Click “Next” to confirm the temperature profile and channel setup, and check the summary to ensure all parameters are correct. Start the Rotor-Gene Q by clicking “Start run”. You will be prompted to enter a file name and to save the run file.
- After the run has started, click on “Finish”.
Windows displaying raw data, temperature, and progress profile will appear.

Table 4. Cycling protocol for use with the Rotor-Gene Q

			Comments
Initial PCR activation step	5 min	95°C	HotStarTaq® Plus DNA Polymerase is activated by this step.
2-step cycling			
Denaturation:	10 s	95°C	
Annealing/extension:	30 s	55°C	Perform fluorescence data collection using the “Green” channel with auto-gain optimization.
Number of cycles:	45		
HRM	2 s	60–85°C; increments of 0.1°C	Optimize gain before melt on all tubes.

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

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