Quick-Start Protocol March 2016

QIAGEN® Multiplex PCR Kit

The QIAGEN Multiplex PCR Kit (cat. nos. 206143 and 206145) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer.

Further information

- *QIAGEN Multiplex PCR Handbook*: **www.qiagen.com/HB-0453**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Choose the most suitable protocol according to amplicon size (Table 1). We have also developed specialized protocols and recommendations for the following advanced applications: PCR assays with more than 10 products, long amplicons (≥1.5 kb), sensitive multiplex PCR assays, transgene detection, detection of genetically modified organisms or microorganisms, qualitative or semi-quantitative gene expression analysis and exon-specific PCR. For more information please refer to the *QIAGEN Multiplex PCR Handbook*.
- The functionality and specificity of all primer pairs should be tested in single reactions before combining them in a multiplex PCR assay.
- The primer mix containing all primers at equimolar concentrations should be prepared in TE, and stored in small aliquots at –20°C to avoid repeated freezing and thawing.
- Primers labeled with fluorescent dyes should always be kept in the dark.
- The sizes of the amplicons must differ sufficiently in order to be able to distinguish them from one another in downstream analysis.
- Always use the cycling conditions specified in this protocol.
- If using an already established multiplex PCR system, use the previously established annealing temperature in combination with the cycling conditions specified in this protocol.
- Annealing time must be 90 s.
- For optimal results, we recommend using primer pairs with a T_m of >68°C.

- Use equal concentrations (0.2 µM) of all primers.
- PCR must start with a heat-activation step of 15 min at 95°C to activate HotStarTaq® DNA Polymerase.
- The QIAGEN Multiplex PCR Kit is provided with Q-Solution® for optional use, which facilitates amplification of templates that have a high degree of secondary structure or that are GC-rich. When using Q-Solution for the first time for a particular primer–template pair, always perform parallel reactions with and without Q-Solution.

Table 1. Protocol selection according to amplicon size

Size and nature of amplicon	Protocol
Up to 1.5 kb	Protocol 1: Standard multiplex PCR
0.05–0.5 kb (e.g., microsatellites or small amplicons)	Protocol 2: Amplification of microsatellite loci or small amplicons using multiplex PCR

Protocol 1: Standard multiplex PCR (up to 1.5 kb)

1. Thaw 2x QIAGEN Multiplex PCR Master Mix, template DNA, RNase-free water, primer mix and Q-solution (optional). Mix thoroughly before use.
2. Prepare a reaction mix according to Table 2.

   Note: The reaction mix typically contains all the components required for multiplex PCR except the template DNA. Prepare a volume of reaction mix 10% greater than that required for the total number of reactions to be performed.

Table 2. Reaction setup for multiplex PCR

Component	Volume/reaction	Final concentration
Reaction mix		
2x QIAGEN Multiplex PCR Master Mix	25 µl	1x*
10x primer mix (2 µM of each primer)	5 µl	0.2 µM of each primer
RNase-free water	Variable	–
Optional: 5x Q-Solution†	10 µl	1x
Template DNA (added at step 4)	Variable	≤1 µg DNA/reaction
Total reaction volume	50 µl	

* Contains 3 mM Mg^{2+}.

† For templates with GC-rich regions or complex secondary structure.

3. Mix the reaction mix gently but thoroughly, for example by pipetting up and down a few times. Dispense appropriate volumes into PCR tubes or plates. It is not necessary to keep samples on ice during reaction setup.
4. Add template DNA (≤1 µg/50 µl reaction) to the individual PCR tubes or wells containing the reaction mix. For multiplex RT-PCR, add an aliquot from the reverse transcriptase reaction. This should not exceed 10% of the final PCR volume.
5. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Table 3.
6. Place the PCR tubes in the thermal cycler and start the cycling program as outlined in Table 3.

   Note: After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer storage.
7. Analyze samples using an appropriate detection system. The optimal amount of PCR product required to give a satisfactory signal with your detection method should be determined individually.

Table 3. Cycling conditions for standard multiplex PCR

Step	Time	Temperature	Comment
Initial heat activation:	15 min	95°C	Activates HotStarTaq DNA Polymerase.
3-step cycling:			
Denaturation	30 s	94°C	Do not exceed this temperature.
Annealing	90 s	57–63°C	Use 60°C as the starting temperature. If the lowest T_m of your primer mixture is below 60°C, use 57°C as the starting temperature.
Extension	90 s	72°C	Optimal for targets up to 1.5 kb in length.*
Number of cycles	30–45		
Final extension:	10 min	72°C	

* For targets longer than 1.5 kb, an extension time of 2 min may improve performance.

Protocol 2: Amplification of microsatellite loci or small amplicons (up to 0.5 kb) using multiplex PCR

1. Carry out steps 1, 2, 3 and 4 of Protocol 1.
2. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Table 4.
3. Place the PCR tubes in the thermal cycler and start the cycling program.

   Note: After amplification, samples can be stored overnight at 2–8°C, or at –20°C for longer storage.
4. Proceed as in step 7 of Protocol 1.

Table 4. Cycling conditions for amplification of microsatellites or short amplicons using multiplex PCR

Step	Time	Temperature	Comment
Initial heat activation:	15 min	95°C	Activates HotStarTaq DNA Polymerase.
3-step cycling:			
Denaturation	30 s	94°C	Do not exceed this temperature.
Annealing	90 s	57–63°C	Use 60°C as the starting temperature. If the lowest T_m of your primer mixture is below 60°C, use 57°C as the starting temperature.
Extension	60 s	72°C	Optimal for targets up to 0.5 kb in length.
Number of cycles	25–40		
Final extension:	30 min*	60°C	

* Allows generation of A-overhangs by HotStarTaq DNA Polymerase required for high-resolution analysis using capillary- or gel-based DNA sequencers.

Scan QR code for handbook.

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