Quick-Start Protocol June 2016

QIAGEN® Multiplex PCR *Plus* Kit

The QIAGEN Multiplex PCR *Plus* Kit (cat. nos. 206151, 206152 and 206155) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer. The 2x Multiplex PCR Master Mix can also be stored at 2–8°C for up to 2 months if not otherwise stated on label.

Further information

- *QIAGEN Multiplex PCR* Plus *Handbook*: **www.qiagen.com/HB-0526**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- We have also evaluated several specialized protocols for the following cases: PCR assays with more than 10 products, PCR of long amplicons (≥1.5 kb), sensitive multiplex PCR assays, transgene detection, detection of genetically modified organisms or microorganisms, qualitative or semiquantitative gene expression analysis and exon-specific PCR. For more information, please refer to the *QIAGEN Multiplex PCR* Plus *Handbook*, which can be found at **www.qiagen.com/handbooks**.
- **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.**
- **Use equal concentrations (0.2 µM) of all primers.**
- **For optimal results, we recommend using primer pairs with a T_m of ≥68°C.**
- Prepare a 10x primer mix, using 2 µM of each primer.
- **Optional**: Q-Solution® can be used for templates that are GC rich (>65%) or have a high degree of secondary structure. If using Q-Solution for the first time, it is important to perform parallel amplification reactions with and without Q-Solution.

- **Optional**: CoralLoad® Dye can be used for easy visualization. Note that CoralLoad Dye must not be used if using capillary sequencers.
- The functionality and specificity of all primer pairs should be tested in single reactions before combining them in a multiplex PCR assay.
- Primers labeled with fluorescent dyes should always be kept in the dark.
- **PCR must start with an activation step of 5 minutes at 95°C.**

Table 1. Protocol selection according to template size

Size of amplicon	Protocol
Up to 1.5 kb	Protocol 1: Multiplex PCR of fragments up to 1.5 kb in length
Up to 500 bp	Protocol 2: Multiplex PCR of fragments up to 500 bp in length

Protocol 1: Multiplex PCR of fragments up to 1.5 kb in length

1. Thaw the 2x Multiplex PCR Master Mix (if stored at –20°C), template DNA, RNAse-free water, Q-Solution (optional), CoralLoad Dye (optional) and the primer mix. Mix the solutions completely 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

Component	Volume/reaction	Final concentration
Reaction mix		
2x Multiplex PCR Master Mix	25 µl	1x
10x primer mix, 2 µM each primer	5 µl	0.2 µM
Optional: Q-Solution, 5x*	5 µl	0.5x
Optional: CoralLoad Dye, 10x†	5 µl	1x
RNase-free water	Variable	–
Template DNA (added at step 4)	Variable	≤300 ng DNA Start with 100 ng DNA
Total volume	50 µl	

* For GC-rich templates. † Do not include if using capillary sequencers for analysis.

3. Mix the reaction mix thoroughly and dispense appropriate volumes into PCR tubes or plates. Mix gently, for example, by pipetting the reaction mixture up and down a few times. Due to the hot start, it is not necessary to keep samples on ice during reaction setup.
4. Add template DNA (≤300 ng /reaction) to the individual PCR tubes containing the reaction mix (see Table 2).
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.

Table 3. Cycling protocol for multiplex PCR of up to 1.5 kb fragments

			Comments
Initial PCR activation step	**5 min**	**95°C**	HotStarTaq *Plus* DNA Polymerase is activated by this step.
3-step cycling:			
Denaturation:	30 s	95°C	
Annealing:	90 s	60°C	If the lowest T_m of your primer mixture is below 60°C, use 57°C as starting annealing temperature.
Extension:	90 s	72°C	Optimal for targets up to 1.5 kb in length.
Number of cycles:	35		35 cycles give sufficient results in most cases. See Table 5 for further recommendations.
Final extension:	10 min	68°C	For analysis on capillary sequencers, a final extension time of 30 min must be used.

7. Analyze samples on an agarose gel or the QIAxcel® Advanced System or the Agilent® 2100 Bioanalyzer. The optimal amount of PCR product required to give a satisfactory signal with your detection method should be determined individually.

Protocol 2: Multiplex PCR of fragments up to 500 bp in length

1. Proceed with steps 1–4 from Protocol 1.
2. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Table 4.
3. Proceed with steps 6–7 from Protocol 1.

Table 4. Cycling protocol for multiplex PCR of up to 500 bp fragments

			Comments
Initial PCR activation step	**5 min**	**95°C**	HotStarTaq *Plus* DNA Polymerase is activated by this step.
3-step cycling:			
Denaturation:	30 s	95°C	
Annealing:	90 s	60°C	If the lowest T_m of your primer mixture is below 60°C, use 57°C as starting annealing temperature.
Extension:	30 s	72°C	Optimal for targets up to 500 bp in length.
Number of cycles:	35		35 cycles give sufficient results in most cases. See Table 5 for further recommendations.
Final extension:	10 min	68°C	For analysis on capillary sequencers, a final extension time of 30 min must be used.

Table 5. Recommendations for template amount and cycle number

Amount of starting template (ng DNA per reaction)	Number of cycles*
100–300	30–35
10–100	35–40
0.1–10	40–45

* If using fluorescently labeled primers and a capillary sequencing instrument as an analysis platform, in general 5 cycles less are recommended.

Scan QR code for handbook.

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