Quick-Start Protocol March 2016

Type-it® Microsatellite PCR Kit

The Type-it Microsatellite PCR Kit (cat. nos. 206241, 206243 and 206246) should be stored immediately upon receipt at –30 to –15°C in a constant-temperature freezer.

Further information

- *Type-it Microsatellite PCR Handbook*: **www.qiagen.com/HB-0266**
- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- This protocol is optimized for use with fluorescent primers and subsequent high-resolution fragment analysis using capillary sequencing instruments. When using other downstream analysis platforms (e.g., agarose gels or the QIAxcel® System), please refer to the *Type-it Microsatellite 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.
- Use equal concentrations (0.2 µM) of all primers.

- Annealing time must be 90 s.
- For optimal results, we recommend using primer pairs with a T_m of ≥68°C.
- PCR must start with a heat-activation step of 5 min at 95°C to activate HotStarTaq® *Plus* DNA Polymerase.
- The Type-it Microsatellite PCR Kit is provided with Q-Solution®, which facilitates amplification of templates that have a high degree of secondary structure or that are GC-rich by modifying the melting behavior of DNA. When using Q-Solution for the first time in a microsatellite PCR assay, always perform parallel reactions with and without Q-Solution.

1. Thaw 2x Type-it 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 1.

   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.
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. Due to the hot start, it is not necessary to keep samples on ice during reaction setup.

Table 1. Reaction setup for the Type-it Microsatellite PCR Kit

Component	Volume/reaction	Final concentration
Reaction mix		
2x Type-it Multiplex PCR Master Mix	12.5 µl	1x*
10x primer mix (2 µM of each primer)	2.5 µl	0.2 µM of each primer
RNase-free water	Variable	–
Optional: 5x Q-Solution†	5 µl	1x
Template DNA (added at step 4)	Variable	≤200 ng DNA/reaction start with 10 ng
Total reaction volume	25 µl	

* Contains 3 mM Mg^{2+}.

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

4. Add template DNA (≤200 ng/25 µl reaction) to the individual PCR tubes or wells containing the reaction mix. See Table 3 for exact values.
5. Program the thermal cycler according to the manufacturer's instructions, using the conditions outlined in Table 2.

Table 2. Cycling conditions

Step	Time	Temperature	Comment
Initial heat activation:	5 min	95°C	Activates HotStarTaq *Plus* DNA Polymerase.
3-step cycling:			
Denaturation	30 s	95°C	
Annealing	90 s	57–63°C	An annealing temperature of 60°C is suitable for most PCR systems. If the lowest T_m of your primer mixture is below 60°C, use 57°C as the starting annealing temperature.
Extension	30 s	72°C	Optimal for targets up to 0.5 kb in length.*
Number of cycles	28		Gives satisfactory results in most cases. The optimal number of cycles depends on the amount of template DNA and the required sensitivity of your detection method. See Table 3 for further recommendations.
Final extension	30 min	60°C	

* For targets longer than 0.5 kb, increase the extension time by 30 s per 0.5 kb.

Table 3. Recommended number of PCR cycles for different template amounts for analysis on capillary sequencing instruments

Amount of starting template (ng DNA per PCR reaction)	Number of cycles
50–200	20–24
10–50	24–28
0.1–10	28–32

6. 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 long-term storage.

7. Analyze the samples using a capillary sequencing instrument.

   Note: Prepare a 1:10 to 1:50 dilution of your PCR products (1:10 is sufficient in most cases) before injecting into a capillary sequencer. Dilute the PCR products in deionized formamide or water. Samples (and size standards; see below) are less stable in water than in deionized formamide. Add up to 1 µl of undiluted sample per reaction.

   Note: A fluorescently labeled size standard must also be added to each sample before analysis. Any appropriate commercially available fluorescently labeled size standard can be used. Follow the supplier's instructions for the amount and handling of the size standard.

8. Perform a denaturation step of 5 min at 95°C before injection into the sequencer.

Scan QR code for handbook.

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