

Technical Note: Multiplex PCR Using the FastStart High Fidelity PCR System

The Roche Applied Science FastStart High Fidelity PCR System is the product of choice for multiplex PCR. As the new Amplification Technical Note “Step-by-Step Protocol for Multiplex PCR with the FastStart High Fidelity PCR System and the PCR Optimization Kit” demonstrates, the system amplifies up to 18 PCR fragments simultaneously after the reaction is optimized with the PCR Optimization Kit.

All these steps can use either DNA or cDNA as target. The cDNA is thereby generated with the Transcriptor First Strand cDNA Synthesis Kit.

The Technical Note offers a detailed protocol for multiplexing starting from DNA or RNA, a part on troubleshooting, nine different multiplex examples including customer data, and it describes the features of the system:

- ➔ Generate up to 18-plex PCR
- ➔ Amplify fragments up to 1.8 kb
- ➔ Detect as little as 0.375 ng of target
- ➔ Set up the PCR at room temperature
- ➔ Quickly and simply optimize $MgCl_2$ concentration and pH using the PCR Optimization Kit

Contact your local sales representative to obtain your personal copy of this Technical Note. It is also available on our website:

www.roche-applied-science.com/pcr

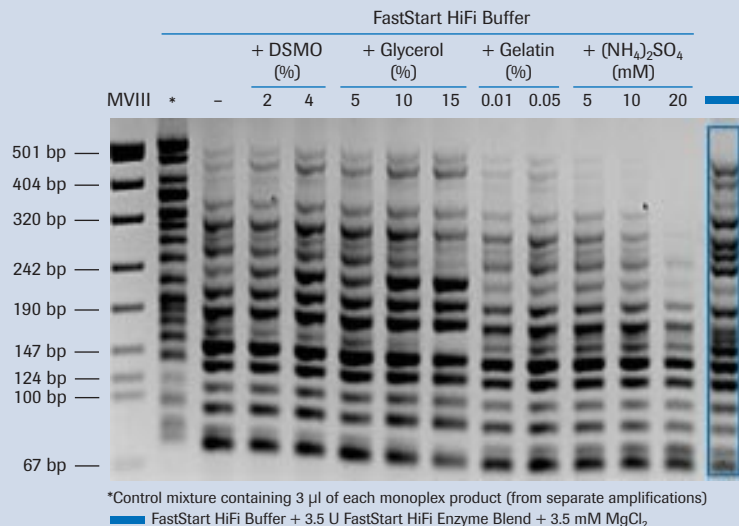


Figure 1: Example from the Technical Note. Multiplex PCR with the best performing PCR buffer (FastStart High Fidelity Buffer) and different additives.

New Technical Note on Multiplex PCR

The Technical Note shows that multiplex PCR can easily be optimized in three simple, straightforward steps, even with a very complex primer set (up to 18 different PCR products, Figure 1). The three optimization steps are:

1. Monoplex PCR of all fragments individually: Starting with amplification of individual primer sets (monoplex) is important, since this step establishes that each single primer pair produces the expected PCR product.
2. Initial optimization of multiplex PCR with buffers from the Roche Applied Science PCR Optimization Kit
3. Final optimization of multiplex PCR with the best-performing PCR buffer and different additives

Product	Pack Size	Cat. No.
FastStart High Fidelity PCR System	125 U (50 reactions)	03 553 426 001
	500 U (2 x 250 U, 200 reactions)	03 553 400 001
	2,500 U (10 x 250 U, 1,000 reactions)	03 553 361 001

