AMPLIQON
PCR ENZYMES & REAGENTS

Tips for successful Fast PCR setup

- *Without the need for fast ramping PCR cyclers and specialized DNA Polymerases*

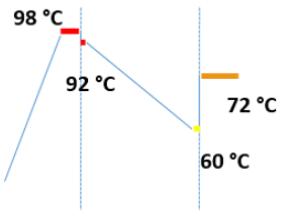
- Start up with our recommended 2-step Fast PCR protocol: 98 °C, 40 sec; then 30 cycles of 92 °C, 2 sec. and 60 °C, 2 sec. and then 72 °C, 20 sec.
- Optimize the Annealing/Extension temperature by performing a temperature gradient.
- Primers must have the highest possible T_m values in the range 58 – 72 °C.*
- At low starting target number, it is recommended to perform 5 – 10 additional cycles.
- If PCR product/amplicon is > 300 bp it may be necessary to increase the hold time of the Annealing/Extension step.
- Sufficient denaturation of for example GC rich templates and other difficult DNA templates is crucial – increase to 95 °C and increase hold time.
- If using TEMPase Hot Start DNA Polymerase the initial denaturation should be 15 min.
- Primer and template quality is important for successful fast PCR protocols.

* *Primers with highest T_m values supports highest annealing temperature → leading to reduced PCR run time, due to shortened ramping time between the Annealing/Extension and Denaturation steps.*

Recommended protocol for 2-step Fast PCR

– Valid for all variants of Ampliqon Taq DNA Polymerases, Taq Master mixes and Taq OptiMix CLEAR

31 min



2-step Fast PCR protocol

PCR program for 2-step Fast PCR – 31min total

Cycler step	Temperature	Duration	Cycles
Initial heating	98 °C	40 sec.	1
Denaturation	92 °C	2 sec.	
Extension*	60 °C	2 sec.	30
Final extension	72 °C	20 sec.	1

* the extension temperature depends on the primer set. For fast PCR choose highest possible Tm values.

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