Colony PCR (Protocol for Thermo Scientific™ Phire™ Hot Start II DNA Polymerase)

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1 Works for me

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ABSTRACT

Thermo Scientific™ Phire™ Hot Start II DNA Polymerase is a novel DNA polymerase designed for use in all routine and high throughput PCR applications. A special DNA binding domain linked to the Phire Hot Start II DNA Polymerase enhances the processivity of the polymerase, enabling short extension times and improved yields. The polymerase is also capable of amplifying long DNA fragments, such as the 7.5 kb genomic DNA used in Thermo Scientific quality control assays. Phire Hot Start II DNA Polymerase provides 2-fold fidelity compared to Taq DNA polymerase. The hot start modification of the polymerase is based on the Affibody™ inactivation method1,2 . It inhibits DNA polymerase activity at ambient temperatures, thus preventing amplification of non-specific products. At polymerization temperatures the Affibody molecule is released, rendering the polymerase fully active. Phire Hot Start II DNA Polymerase generates blunt ends in the amplification products. It does not possess the 5'→3' exonuclease activity needed for hydrolysis experiments.

GUIDELINES

The annealing rules are different from many common DNA polymerases (such as Taq DNA polymerases).

Use 10–15 s/kb for extension.

Phire Hot Start II DNA Polymerase produces blunt end DNA products.

MATERIALS

NAME	CATALOG #	VENDOR
Phire Hot Start II PCR Master Mix	F125S	Thermo Fisher

SAFETY WARNINGS

Wear laboratory coat and glasses during the whole procedure.

1 Add all components in a 250 µL tube making up to a 20 or 50 µl reaction.

'Pro-tip': Carefully mix and centrifuge all tubes before opening to ensure homogeneity and improve recovery. When using Phire Hot Start II PCR Master Mix, it is not necessary to perform the PCR setup on ice.

'Note': Due to the unique nature of Phire Hot Start II DNA Polymerase, optimal reaction conditions may differ from standard enzyme protocols.

'Pro-tip':Phire Hot Start II DNA Polymerase tends to work better at elevated denaturation and annealing temperatures due to higher salt concentrations in its buffer.

Add items in this order:

Components	20 µL reaction	50 µL reaction	Final concentration
H2O	add to 20 µL	add to 50 µL	
2X Phire Hot Start II PCR Master Mix	10 µL	25 µL	1X
Forward primer *	X µL	X µL	0.5 µM
Reverse primer *	X µL	X µL	0.5 µM
Template DNA	X µL	X µL	
DMSO**, optional	0.6 µL	1.5 µL	3%

* The recommendation for final primer concentration is 0.5 µM, but it can be varied in a range of 0.2–1.0 µM, if needed.

** Addition of DMSO is recommended for GC-rich amplicons. DMSO is not recommended for amplicons with very low GC %.

2 Gently mix the PCR reactions and transfer the tubes to a thermocycler. Thermocycling conditions for a routine PCR:

Step	Temperature	Time	Cycles
Initial Denaturation	98ºC	30 s	1
Denaturation	98ºC	5 s	25-35
Annealing*	XºC	5 s	
Extension**	72ºC	10-15 s/kb	
Final Extension	72ºC	1 min	1
Hold	4ºC		

* The optimal annealing temperature for Phire Hot Start II DNA Polymerase may be significantly different than annealing temperature with other DNA polymerases. Always use the Tm calculator and instructions from the supplier to determine the Tm values of the primers and optimal annealing temperature. As a basic rule, for primers > 20 nt, anneal for 5 seconds at a Tm +3 °C of the lower Tm primer. For primers ≤ 20 nt, use an annealing temperature equal to the Tm of the lower Tm primer. If necessary, use a temperature gradient to find the optimal annealing temperature for each template-primer pair combination.

** The extension should be performed at 72 °C. Extension time of 10 seconds per 1 kb is recommended for most templates. However, higher yields may be obtained using extension time of 15 s/kb with challenging primer template pairs.