Direct PCR solutions

Thermo Scientific™ Direct PCR solutions

- Take a small piece of sample to minimize the amount of inhibitors in the PCR reaction; a diameter of 0.3–0.5 mm is recommended.
- Go to **thermofisher.com/directpcr** to see a list of tested tissues and recommendations for sample sizes.
- Add the sample directly to a PCR mix, not an empty tube, to minimize sample degradation.

Setting up your PCR reactions

Component		20 µL rxn	50 µL rxn*	Final conc.
Direct PCR master mix (2X)		10 µL	25 µL	1X
Primer A		X µL	X µL	0.5 µM
Primer B		X µL	X µL	0.5 µM
Sample protocol				
	Direct	–	Amount depends on the sample**	–
	Dilution	0.5–1 µL	2.5 µL	
H_2O		To 20 µL total	To 50 µL total	

* 50 µL reaction volume is recommended for the Direct protocol.

** 0.5 mm punch or a comparable small tissue sample.

PCR cycling conditions

	2-step protocol			3-step protocol			
		Phusion master mix Cat. No. F-175S/F-175L	Phire master mix Cat. No. F-170S/F-170L F-160S/F-160L		Phusion master mix Cat. No. F-175S/F-175L	Phire master mix Cat. No. F-170S/F-170L F-160S/F-160L	
Cycle step	Temp.	Time	Time	Temp.	Time	Time	Cycles
Cell lysis	98°C	5 min	5 min	98°C	5 min	5 min	1
Denaturation	98°C	1 sec	5 sec	98°C	1 sec	5 sec	35–40
Annealing	–	–	–	X°C†	5 sec	5 sec	
Extension	72°C	15–30 sec/kb	<20 sec for ≤1 kb, 20 sec/kb for >1 kb	72°C	15–30 sec/kb	<20 sec for ≤1 kb, 20 sec/kb for >1 kb	
Final extension	72°C 4°C	1 min Hold	1 min Hold	72°C 4°C	1 min Hold	1 min Hold	1

† Annealing temperature depends on the primers used for each reaction; determine the optimal annealing temperature using our T_m calculator at **thermofisher.com/tmcalculator**

ThermoFisher SCIENTIFIC

thermo scientific

Dilution and storage protocol

The dilution and storage protocol is recommended when:

- Amplifying longer (>1 kb) fragments
- Setting up multiple PCR reactions from one sample, or when optimization is required
- Performing challenging applications where template amount is critical and titration is needed

Samples in dilution buffer can be stored for up to 4 weeks at different temperatures (–20°C, +4°C, or room temperature) before using them in PCR.

Control reactions

- Perform a positive control reaction with purified DNA to determine if the PCR conditions are optimal. If this control fails, the PCR conditions should be optimized further.
- Use the supplied control primers with your sample to determine optimal sample size and compatibility with our Direct PCR approach.

Optimizing cycling conditions

- Use the T_m calculator (**thermofisher.com/tmcalculator**) to determine the T_m values of primers and optimal annealing temperature for Thermo Scientific™ Phusion™ and Phire™ Direct PCR master mixes, which are different from other *Taq*-based DNA polymerases.
- For multiple primer pairs, use a temperature gradient to find the optimal annealing temperature. The annealing gradient should extend up to the extension temperature (2-step PCR).
- For high-T_m primer pairs (T_m at least 69–72°C), use 2-step cycling without an annealing step.

Note

Thermo Scientific™ DNARelease™ Additive is required when PCR is performed directly from certain tissue samples using the Direct protocol. Cell debris present in these PCR reactions can cause DNA molecules to get trapped in the agarose gel wells. DNARelease Additive helps to eliminate this problem.

Find out more at **thermofisher.com/directpcr**

ThermoFisher SCIENTIFIC