

# Direct PCR protocol for mouse tissue using Phire<sup>®</sup> Hot Start DNA Polymerase



Application protocol

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There are two alternative protocols available, (1) direct and (2) dilution protocol.

## 1. Direct protocol

Take as small a sample from the mouse tissue as possible for each 50 µl PCR reaction. Ear tissue is preferred, but tails can also be used. Recommended amounts of tissue:

- 0.5 mm punch disc cut by using a Harris Uni-Core<sup>™</sup> and a compatible Cutting Mat<sup>™</sup> (available from Finnzymes)
- Alternatively, less than 1 mm section of tail (cut with a clean scalpel).

Use the recommended protocol for Phire<sup>®</sup> Hot Start DNA Polymerase, but increase the initial denaturation time at 98°C to 5 minutes and use a minimum of 20 seconds in the extension step. Use 40 cycles.

Table 1. Cycling conditions.

Cycle step	Temperature	Time	Number of cycles
Initial denaturation	98°C	5 min	1
Denaturation	98°C	5 s	40
Annealing	X°C	5 s*	
Extension	72°C	20 s ≤ 1 kb 20 s/kb > 1 kb	
Final extension	72°C 4°C	1 min hold	1

\* Depending on the primers; see instructions below and the product manual

The optimal annealing temperature depends on the primers. With Phire Hot Start DNA Polymerase, the optimal annealing temperature is typically over 60°C, but some primers may require lower temperatures. Temperature gradient is a useful tool in finding an optimal annealing temperature. It is recommended to use Finnzymes' Tm calculator to set up the protocol.

After PCR, spin down the reactions and take the supernatant to gel electrophoresis. In Direct PCR from mouse tissue, it is necessary to use the DNAResult<sup>™</sup> Additive (F-355) in gel loading dye to improve PCR product migration in the gel (see the application note for mouse tissue).

## 2. Dilution protocol

Place a small piece of tissue (2 mm ear punch or 1 mm tail section) into 20 µl of 1x TE buffer (pH 8.0) and add 0.5 µl of DNAResult Additive (F-355). After vortexing for 15 seconds, incubate at 75°C for 5 minutes and then at 96°C for 2 minutes. In some cases, it can be helpful to crush the tissue e.g. with a pipette tip before incubation.

After centrifugation, remove the supernatant and use 0.5–4 µl of supernatant as template in 20 µl PCR reaction with Phire Hot Start DNA Polymerase. Use the same PCR protocol and post-PCR treatments as mentioned in Section 1.

## General notes

1. As in all Direct PCR applications, it is highly recommended to include a positive control (purified DNA) to ensure that the PCR reaction conditions are optimal. Also a negative control without DNA template is recommended.
2. Use as small a sample of the tissue as possible in Direct PCR. For longer amplicons, smaller tissue samples give more consistent results.

Finnzymes' Direct PCR allows amplification of DNA directly from various starting materials such as blood, mouse ear and tail tissues, plants, and FFPE tissue samples. For more information about the Direct PCR products and protocols, please visit [www.finnzymes.com/directpcr](http://www.finnzymes.com/directpcr).