Thermo Scientific Phire Animal Tissue Direct PCR Kit

F-140, 200 PCR reactions 50 µl each

The Dilution Buffer is sufficient for 250 dilution reactions 20 µl each.

Store at -20°C

1. Introduction

Thermo Scientific Phire Animal Tissue Direct PCR Kit is designed to perform PCR directly from non-fixed animal derived tissue samples with no prior DNA purification. Tissues such as mouse ear and tail, zebrafish fin and *Drosophila* are suitable starting materials. The samples can either be fresh or stored at –20°C. A list of animal tissues tested with this kit is available at www.thermoscientific.com/directpcr.

The Phire® Animal Tissue Direct PCR Kit employs Phire Hot Start II DNA Polymerase, a specially engineered enzyme with a DNA-binding domain that enhances the processivity of the polymerase. Phire Hot Start II DNA Polymerase also exhibits extremely high resistance to many PCR inhibitors found in animal tissues.

The Phire Animal Tissue Direct PCR Kit contains reagents and tools for two alternative protocols: direct and dilution protocols. See Section 4.3 for information about protocol options.

A 0.5 mm Harris Uni-Core™ puncher and a compatible Harris Cutting Mat™ are included for convenient sample handling.

In addition, the kit includes a pair of universal control primers that is compatible with a number of vertebrate species (see Section 7). For other species such as *Drosophila* and zebrafish, validated control primer sequences are available at www.thermoscientific.com/directpcr. The kit is recommended for end point PCR protocols.

IMPORTANT NOTES

- Use 98°C for denaturation.
- The annealing rules are different from many common DNA polymerases (such as *Taq* DNA polymerases). Read Section 6.3 carefully.
- Use 50 µl reaction volume for direct protocols.
- Add the sample directly into a PCR reaction instead of an empty tube.
- Clean the sampling tool between each sample (see 4.2).
- The dilution protocol (see 4.3) is recommended:
  - When working with new sample materials or a new primer pair.
  - With difficult samples or long amplicons.
  - When performing multiple reactions from the same sample.

2. Package information

Component	F-140
Phire Hot Start II DNA Polymerase	200 µl
2x Phire Animal Tissue PCR Buffer (includes dNTPs and $MgCl_2$)	5 x 1 ml
Universal control primer mix (25 µM each)	40 µl
Dilution Buffer	5 ml
DNARelease Additive	3 x 100 µl
Gel loading dye	3 x 1 ml
Harris Uni-Core™ 0.5 mm	1
Harris Cutting Mat™	1

Other materials needed, but not included in the kit:
2 % sodium hypochlorite solution.

Material safety data sheet (MSDS) is available at www.thermoscientific.com/fzmsds.

3. Guidelines for PCR

Carefully mix and spin down all tubes before opening to ensure homogeneity and improve recovery. The PCR setup can be performed at room temperature. **Always add the sample last to the reaction.** Read Section 4 carefully for sampling guidelines.

Table 1. Pipetting instructions.

Component	20 µl reaction	50 µl reaction*	Final conc.
H_2O	add to 20 µl	add to 50 µl	
2x Phire Animal Tissue PCR Buffer	10 µl	25 µl	1x
primer A	x µl	x µl	0.5 µM
primer B	x µl	x µl	0.5 µM
Phire Hot Start II DNA Polymerase	0.4 µl	1 µl	
Sample (see Section 4) Direct protocol: Dilution protocol:	 – 1 µl	 Amount depends on the sample** 2.5 µl	

* 50 µl reaction volume is recommended for the direct protocol.
**0.5 mm punch or a small sample of animal tissue (see www.thermoscientific.com/directpcr)

Table 2. Cycling protocol.

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	98°C	5 min	98°C	5 min	1
Denaturation Annealing (See 6.3) Extension (See 6.4)	98°C - 72°C	5 s - 20 s ≤ 1 kb 20 s/kb > 1 kb	98°C X°C 72°C	5 s 5 s 20 s ≤ 1 kb 20 s/kb > 1 kb	40
Final extension	72°C 4°C	1 min hold	72°C 4°C	1 min hold	1

4. Guidelines for sample handling

To obtain small and uniform samples, we recommend using the Harris tools provided in the kit. The Harris Uni-Core™ may be disposed of after use or cleaned and reused up to 500 times, depending on the thickness and firmness of the sample material. If the puncher is to be reused, it is very important to clean the cutting edge properly to prevent cross-contamination between samples (see Section 4.2 for cleaning instructions). The Harris Cutting Mat™ provides the best possible cutting surface for Harris Uni-Core™. It is made of inert self-healing material and has two cutting surfaces. The Cutting Mat can be reused several hundred times, but it should be cleaned between each sample to prevent cross-contamination (see Section 4.2). The Harris tools are also available separately from Thermo Scientific.

4.1 The use of Harris Uni-Core™

Note: The cutting edge of Harris Uni-Core™ is very sharp. Therefore, special attention is required when working with this puncher. Please follow the instructions below.

1. Place the sample on the Harris Cutting Mat™.
2. Remove the protective cap from the cutting edge of the Harris Uni-Core by twisting it gently or flipping it off using your thumb.
3. Holding the puncher firmly, push the cutting edge downward into the sample and rotate the puncher in opposite directions until it cuts through the sample. Only very gentle downward pressure is required. **Do not press the plunger while cutting.**
4. Lift the puncher away from the sample and press the plunger to eject the punch disc into a PCR reaction. Make sure that the sample drops into the PCR solution and does not stick to the tube walls.
5. Clean both the Harris Uni-Core™ and the Cutting Mat after every sample as described in Section 4.2.

A video showing the sampling and cleaning procedure is available at www.thermoscientific.com/directpcr.

4.2 Cleaning the sampling tools

To prevent cross-contamination between samples, it is important to clean the cutting edge of the Harris Uni-Core™ between each sample. Dip the cutting edge into 2 % sodium hypochlorite* (NaClO) solution and press the plunger up and down a few times. After rinsing, wipe the tip with a clean paper towel. The Cutting Mat should also be rinsed with 2 % sodium hypochlorite solution after each sampling.

It is recommended to include a negative control without DNA template in all assays. To monitor cross-contamination, the cleaned puncher can be dipped into the negative control sample (see Section 7.3). For more information, refer to the application protocol about avoiding cross-contamination when using the Harris Uni-Core™ (available at www.thermoscientific.com/directpcr).

*Never mix sodium hypochlorite with acids or ammonia-based cleaners.

4.3 Sample types and protocols

This kit is optimized for various animal tissue samples. **Please visit www.thermoscientific.com/directpcr to see a list of tested animal tissues and recommendations for sample sizes.** With a few exceptions, both direct and dilution protocols are compatible with all sample types and applications. However, when amplifying longer fragments (e.g. > 500 bp from fish fin tissue or > 1 kb from other tissues) the dilution protocol is recommended. The dilution protocol is also useful when multiple PCR reactions are performed from the same sample or in some challenging applications where template amount is critical and titration is needed. When working with new sample materials or a new primer pair, start with the dilution protocol, as it allows several PCR reactions to be performed from the same sample if optimization is required.

4.3.1 Direct protocol

1. Take a sample from animal tissue by using the 0.5 mm Harris Uni-Core™ puncher supported by the Cutting Mat. Alternatively, use a sterile scalpel to cut a very small piece of tissue (e.g. one *Drosophila* wing).
2. Place the sample directly into the PCR reaction (50 µl of volume). It is recommended to place the sample into the liquid rather than into an empty tube. Make sure that you see the sample in the solution.

Gel electrophoresis

In the direct protocol, it is recommended to use the DNARelease™ Additive in the loading buffer for gel electrophoresis as otherwise cell debris present in the PCR products can cause DNA fragments to get trapped in the agarose gel wells. Mix 100 µl of DNARelease Additive with 1 ml of gel loading dye. The dilution is stable for 2 weeks at +4°C. For long term use, store the dilution at –20°C. Before agarose gel electrophoresis, add 15 µl of the pre-mixed gel loading dye into a 50 µl PCR reaction.

4.3.2 Dilution protocol

Before beginning, warm a heat block to 98°C.

1. Place the tissue sample into 20 µl of Dilution Buffer.
2. Add 0.5 µl of DNARelease Additive. Mix by vortexing the tube briefly, and spin down the solution. If a larger sample is used, adjust the volume of the Dilution Buffer and DNARelease Additive accordingly. Make sure the sample is covered with the solution.
3. Incubate the reaction for 2–5 minutes at room temperature and then place the tube into the pre-heated (98°C) block for 2 minutes.
4. Spin down the remaining tissue and store the supernatant at –20°C if not used immediately.

Usually 1 µl of supernatant is sufficient for a 20 µl PCR reaction. In some cases the supernatant may have to be diluted 1:10 or 1:100, or the PCR reaction performed in a 50 µl volume. In the dilution protocol, it is not necessary to use the DNARelease Additive in the gel loading dye.

5. Notes about reaction components

5.1 Enzyme

Phire Hot Start II DNA Polymerase possesses the following activities: 5´→3´ DNA polymerase activity and a weak 3´→5´ exonuclease activity. When cloning fragments amplified with Phire Hot Start II DNA Polymerase blunt end cloning is recommended. If TA cloning is required, it can be performed by adding A overhangs to the blunt PCR product with Thermo Scientific *Taq* DNA Polymerase, for example (protocol available at www.thermoscientific.com/pcrcloning).

5.2 Phire Animal Tissue PCR Buffer

The 2x Phire Animal Tissue PCR Buffer has been optimized for Direct PCR from animal tissues. It contains the dNTPs and provides 1.5 mM $MgCl_2$ concentration in the final reaction.

5.3 Dilution Buffer

The Dilution Buffer has been optimized to release DNA from a wide variety of different animal tissues when supplemented with DNARelease Additive (see Section 5.4). This buffer is also suitable for storing the DNA sample for short periods of time at +4°C. For long term storage, it is recommended to transfer the supernatant into a new tube and store at –20°C.

5.4. DNARelease Additive

DNARelease Additive is required in the gel loading dye when PCR is performed directly from certain tissue samples using the direct protocol. Cell debris present in these PCR products can cause DNA fragments to get trapped in the agarose gel wells. DNARelease Additive eliminates this problem. DNARelease Additive is also used in the dilution protocol to improve the release of DNA from the tissue sample.

5.5 Primers

The recommendation for the final primer concentration is 0.5 µM. The results from primer Tm calculations can vary significantly depending on the method used. Always use the Tm calculator and instructions on our website www.thermoscientific.com/pcrwebtools to determine the Tm values of primers and optimal annealing temperature.

6. Notes about cycling conditions

6.1 Initial denaturation

In Direct PCR protocols, the initial denaturation step is extended to 5 minutes to allow the lysis of cells, making genomic DNA available for PCR.

6.2 Denaturation

Keep the denaturation time as short as possible. Usually 5 seconds at 98°C is enough for most templates. Note that the denaturation time and temperature may vary depending on the ramp rate and temperature control mode of the thermal cycler.

6.3 Primer annealing

Note that the optimal annealing temperature for Phire Hot Start II DNA Polymerase may differ significantly from that of *Taq*-based polymerases. **Always use the Tm calculator and instructions on Thermo Scientific website www.thermoscientific.com/pcrwebtools to determine the Tm values of 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.** In some cases, it may be helpful to use a temperature gradient to find the optimal annealing temperature for each template-primer pair combination. The annealing gradient should extend up to the extension temperature (two-step PCR). Two-step cycling without an annealing step is recommended for high-Tm primer pairs (Tm at least 69–72°C).

6.4 Extension

The extension is performed at 72°C. The recommended extension time is 20 seconds for amplicons ≤ 1 kb, and 20 s/kb for amplicons >1 kb.

7. Control reactions

7.1 Direct PCR control reaction using the control primer mix

When using mammalian tissue samples (e.g. mouse tissue), we recommend performing Direct PCR control reactions with both direct and dilution protocols using the control primers supplied with this kit. As a template, use the same tissue material as in the actual experiment. The universal control primer mix contains degenerate primers that amplify a 237 bp fragment of mammalian genomic DNA. The amplified region is a highly conserved non-coding region upstream of the SOX21 gene,[1] and the primers are designed to amplify this region from a wide range of vertebrate species.

Each primer concentration is 25 µM.

Primer #1 (24-mer)
5´- AGCCCTTGGGGASTTGAATTGCTG -3´
Melting point: 73.5°C

Primer #2 (27-mer)
5´- GCACTCCAGAGGACAGCRGTGTCAATA -3´
Melting point: 72.2°C (R=A), 75.3°C (R=G)

Please note that these control primers are not compatible with fish or insect samples. The recommended control primer sequences for *Drosophila* and zebrafish are available at www.thermoscientific.com/directpcr.

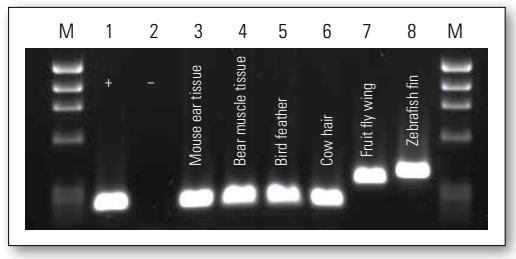
Table 3. Pipetting instructions for control reactions.

Component	20 µl reaction	50 µl reaction*	Final conc.
H_2O	add to 20 µl	add to 50 µl	
2x Phire Animal Tissue PCR Buffer	10 µl	25 µl	1x
Universal control primer mix	0.4 µl	1 µl	0.5 µM
Phire Hot Start II DNA Polymerase	0.4 µl	1 µl	
Sample/Direct protocol: Sample/Dilution protocol:	– 1 µl	Amount depends on the sample** 2.5 µl	

*50 µl volume is recommended for direct protocol
**0.5 mm punch or a small sample of animal tissue (see www.thermoscientific.com/directpcr)

Table 4. Cycling instructions for control reactions using primers included in the kit.

Cycle step	Temp.	Time	Cycles
Initial denaturation	98°C	5 min.	1
Denaturation Annealing/Extension	98°C 72°C	5 s 20 s	40
Final extension	72°C 4°C	1 min hold	1



Amplification of the control DNA fragment from various animal tissues. A small sample of tissue was placed directly into a 50 µl PCR reaction. The fragments in lanes 3–6 were amplified with the control primers included in the kit. Fragments in lanes 7 and 8 were amplified using primers whose sequences are available on www.thermoscientific.com/directpcr. + denotes the positive control reaction with purified DNA and – denotes the no-template control.

7.2 Positive control reaction with purified DNA

When optimizing the reactions, it is recommended to perform a positive control with purified DNA to ensure that the PCR conditions are optimal. If the positive control with purified DNA fails, the PCR conditions should be optimized before continuing further.

7.3. Negative control

It is recommended to add a no-template control to all Direct PCR assays. To monitor the efficiency of cleaning the Harris Uni-Core™, the cleaned tool can be dipped into the negative control sample. A second negative control performed without dipping the sampling tool is recommended to control for other sources of contamination.

8. Troubleshooting

No product at all or low yield

General:
If the positive control reaction with purified DNA using your own primers does not yield a product:
- Make sure the pipetting and cycling protocols were performed as recommended.
- Check primer design.
- Optimize annealing temperature (run a temperature gradient).
- Titrate template amount.
- Optimize denaturation time.
- Increase extension time.

Direct protocol:
If the actual samples yield no product, but the positive control reaction with purified DNA using your own primers and Direct PCR control reaction are working:
- Make sure to add DNARelease Additive to gel loading dye.
- Use a smaller punch (e.g. Harris Uni-Core™ 0.35 mm, F-180).
- Use the dilution protocol.

Dilution protocol:
If the actual samples yield no product, but the positive control reaction with purified DNA using your own primers and Direct PCR control reaction are working:
- Dilute the supernatant 1:10 or 1:100 with H_2O or TE buffer, and use 1 µl as a template in PCR.
- Make sure to perform the 2-minute incubation at 98°C (see 4.3.2, step 3).
- Try incubating the dilution reaction at elevated temperature (up to 65°C) instead of room temperature.
- Try crushing the sample in the tube.

Non-specific products - High molecular weight smears

- Increase the annealing temperature or perform temperature gradient.
- Reduce the total number of cycles.
- Decrease primer concentration.
- Shorten extension time.
- Design new primers.

Non-specific products - Low molecular weight discrete bands

- Increase the annealing temperature or perform a temperature gradient PCR.
- Shorten extension time.
- Decrease primer concentration.
- Reduce the total number of cycles.
- Design new primers.

9. References

1. Woolfe A. *et al.* (2005) *PLoS Biology* 3: 116–130.

Technical support

US: techservice.genomics@thermofisher.com
Europe, Asia, Rest of World:
techservice.emea.genomics@thermofisher.com

Web: www.thermoscientific.com/pcr
Tm-calculator: www.thermoscientific.com/pcrwebtools

Shipping and storage

Phire Animal Tissue Direct PCR Kit is shipped on gel ice. Upon arrival, store the components at –20°C. The Dilution Buffer can also be stored at +4°C once it is thawed.

Product use limitation

This product has been developed and is sold exclusively for research purposes and *in vitro* use only. This product has not been tested for use in diagnostics or drug development, nor are they suitable for administration to humans or animals.

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