# Thermo Scientific Phire Tissue Direct PCR Master Mix

#F-170
Lot 00000000
Store at -20 °C

## 1. Introduction

Thermo Scientific™ Phire™ Tissue Direct PCR Master Mix is designed to perform PCR directly from tissue samples with no prior DNA purification. Tissues such as mouse ear and tail, zebrafish scale and muscle, *Drosophila*, human hair are suitable starting materials. The samples can either be fresh or stored at –70 °C. A list of tissues tested with this Master Mix is available at www.thermoscientific.com/directpcr.

The Phire Tissue Direct PCR Master Mix 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 tissues. The Phire Tissue Direct PCR Master Mix contains reagents for two alternative protocols: Direct and Dilution & Storage. See Section 6 for information about protocol options. In addition, the kit includes a pair of universal control primers that is compatible with a number of vertebrate species (see section 10). For other species such as *Drosophila* and zebrafish, validated control primer sequences are available at www.thermoscientific.com/directpcr. The Master Mix is recommended for end point PCR protocols and it contains premixed gel loading dye which allows direct sample loading on the gel. The loading dye in the Master Mix does not interfere with PCR performance and is compatible with downstream applications such as DNA sequencing, ligation and restriction digestion. For applications that require PCR product analysis by absorbance or fluorescence excitation, we recommend purifying the PCR product prior to analysis or using the Phire Animal Tissue Direct PCR Kit (F-140) or Phusion Human Specimen Direct PCR Kit (F-150) depending on the sample.

## 2. Package information

| Component | #F-170S 100 rxns | #F-170L 500 rxns |
|---|---|---|
| 2x Phire Tissue Direct PCR Master Mix | 2 × 1.25 mL | 10 × 1.25 mL |
| Dilution Buffer | 5 mL | 2 × 12.5 mL |
| DNARelease Additive | 3 × 100 µL | 1.3 mL |
| Universal control primer mix (25 µM each) | 40 µL | 40 µL |
| Water, nuclease- free | 2 x 1.25 mL | 10 x 1.25 mL |
| O'GeneRuler Express DNA Ladder | 100 applications (50 µg) | |

## 3. Important notes

- Primer annealing temperatures for Phire are different from many common DNA polymerases (such as Taq DNA polymerases). Read Section 9.3 carefully.
- Use 98° C for denaturation.
- Use 50 µL reaction volume for direct protocols
- Add the sample directly into a PCR reaction instead of an empty tube.
- The Dilution & Storage protocol (see Section 6) 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.

## 4. 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 5 carefully for sampling guidelines.

### 4.1 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.

### 4.2 Negative control

It is recommended to add a no-template control to all Direct PCR assays.

Table 1. Pipetting instructions

| Component | 20 µL rxn | 50 µL rxn* | Final conc. |
|---|---|---|---|
| $H_2O$ | add to 20 µL | add to 50 µL | - |
| 2X Phire Tissue Direct PCR Master Mix | 10 µL | 25 µL | 1X |
| Primer A | X µL | X µL | 0.5 µM |
| Primer B | X µL | X µL | 0.5 µM |
| Sample (see Section 6) Direct protocol: | - | Amount depends on the sample** | - |
| Dilution & Storage protocol: | 0.5-1 µL | 2.5 µL | |

*50 µL reaction volume is recommended for the direct protocol.
**0.5 mm punch or a small sample of 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 | 98 °C | 5 s | 98 °C | 5 s | 35-40 |
| Annealing (see 9.3) | - | - | X °C | 5 s | |
| Extension (see 9.4) | 72 °C | 20 s ≤1 kb<br>20 s/kb >1 kb | 72 °C | 20 s ≤1 kb<br>20 s/kb >1 kb | |
| Final Extension | 72 °C<br>4 °C | 1 min<br>hold | 72 °C<br>4 °C | 1 min<br>hold | 1 |

## 5. Guidelines for sample handling

To obtain small and uniform samples, we recommend using Harris Uni-Core™ tools. 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. 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. Other ways to take a sample is by cutting with scalpel to obtain 0.50 mm sample.Scalpel and cutting mat must be cleaned properly to prevent crosscontamination between samples.

## 6. Choosing the protocol

This Master Mix is optimized for various tissue samples. Please visit www.thermoscientific.com/directpcr to see a list of tested tissues and recommendations for sample sizes. With a few exceptions, both Direct and Dilution & Storage protocols are compatible with all sample types and applications. However, when amplifying longer fragments (e.g. > 500 bp from fish tissue or > 1 kb from other tissues) the Dilution & Storage protocol is recommended. The Dilution & Storage 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 & Storage protocol, as it allows several PCR reactions to be performed from the same sample if optimization is required. Samples in Dilution Buffer can be stored for up to 4 weeks in different temperatures (-20° C, +4 °C or room temperature) before using in PCR.

### 6.1 Solid samples

**Animal tissues**

1. **Direct protocol:** Take a sample of 0.5 mm in diameter from tissue with a sterile scalpel (or small peace, e.g. one *Drosophila* leg ) or by using the Harris Uni-Core puncher. 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.
2. **Dilution & storage protocol:** Before beginning, warm a heat block to 98 °C. Place the tissue sample into 20 µL of Dilution Buffer. 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. 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. 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.

**Buccal swabs (e.g. nylon flocked swab)**

1. **Direct protocol:** For Nylon flocked swabs take a 0.5 mm punch (or similar) and place it directly into a 50 µL PCR reaction. In some cases reducing the punch size to 0.35 mm may improve the results.
2. **Dilution & storage protocol:** Place the buccal swab tip into a 1.5 ml tube containing 50 µL Dilution Buffer, 1.5 µL DNARelease Additive and 250 µL TE, pH 8. Rotate the swab 5–10 times before removing it from the tube by gently pressing the brush against the side of the tube. Mix by vortexing and spin down. Incubate at 98 °C for 2 minutes, spin down again, and use 0.5 µL of the supernatant as a template for a 20 µL PCR reaction. Note: Swabbing technique and storage conditions (not thoroughly dried) may cause yield variation.

**Hair**

1. **Direct protocol:** Take 2 to 5 hair bulbs and place them directly into a 50 µl PCR reaction.
2. **Dilution & storage protocol:** Place 2 to 5 hair bulbs in a tube that contains 20 µL Dilution Buffer and 0.5 µL DNARelease Additive. Make sure the sample is covered with the solution. Mix by vortexing and spin down. Incubate for 2–5 minutes at room temperature followed by 2 minutes at 98 °C. Spin down again, and use 0.5 µL of the supernatant as a template in a 20 µL PCR reaction.

**Teeth**

1. **Direct protocol:** Place a sample of tooth approximately this size (•) directly into a 50 µL PCR reaction.
2. **Dilution & storage protocol**: Place an approximately 13–15 mg sample of tooth in a tube that contains 50 µL Dilution Buffer and 1.5 µL DNARelease Additive. Make sure the sample is covered with the solution. Mix by vortexing and spin down. Incubate for 2–5 minutes at room temperature followed by 2 minutes at 98 °C. Spin down again, and use 0.5 µL of the supernatant as a template in a 20 µL PCR reaction. **Note:** Using finely crushed sample may yield better results, e.g. improved sensitivity. The sample can be crushed for example by grinding in liquid nitrogen with a mortar and pestle or a homogenizer.

**Skin biopsies (non-fixed)**

1. **Direct protocol:** take a 0.5 mm sample and place it directly into a 50 µL PCR reaction. In some cases, increasing the reaction volume to 50 µL or reducing the punch size to 0.35 mm may improve the results.
2. **Dilution & storage protocol:** Place a 2 mm punch sample into a tube containing 20 µL Dilution Buffer and 0.5 µL DNARelease Additive. Make sure the sample is covered with the solution. Mix by vortexing and spin down. Incubate for 2–5 minutes at room temperature, followed by 2 minutes at 98 °C. Spin down again, and use 0.5 µL of the supernatant as a template in a 20 µL PCR reaction.

**Fingernails**

1. **Direct protocol:** Place a small nail sample (approximately 1 x 2 mm, in other words < 1 mg) directly into a 50 µL PCR reaction.
2. **Dilution & storage protocol:** Place an approximately 7 mg nail sample in a tube that contains 50 µL Dilution Buffer and 1.5 µL DNARelease Additive. Make sure the sample is covered with the solution. Mix by vortexing and spin down. Incubate for 2–5 minutes at room temperature followed by 2 minutes at 98 °C. Spin down again, and use 0.5 µL of the supernatant as a template in a 20 µL PCR reaction. the supernatant as a template in a 20 µL PCR reaction. **Note:** Using finely diced sample may yield better results, e.g. improved sensitivity.

**Formalin-fixed paraffin-embedded (FFPE) tissues**

1. **Direct protocol:** Not recommended
2. **Dilution & storage protocol:**
   **•FFPE tissue section**

   Place one 7–10 µm thick FFPE human tissue section into a tube containing 50 µL Dilution Buffer, 1.5 µL of DNARelease Additive and 50 µL TE, pH 8.* Crush with a pipette tip and spin down. Make sure the sample is covered with the solution. Incubate for 1 hour at 60 °C followed by 10 minutes at 98 °C. After cooling and centrifuging (16 000 x g, 2 min), transfer the supernatant into a new tube. Use 0.5 µL of the supernatant as a template in a 20 µL PCR reaction.

   In some cases, if the amount of tissue debris or DNA in the supernatant is very high, the supernatant may need to be diluted. Make a 1:10 or 1:100 dilution in $H_2O$ or TE buffer and use 0.5 µL of the dilution as a template in a 20 µL PCR reaction.
   **•FFPE tissue on a microscope slide (unstained)**

   Prepare a 1:1 mixture of Dilution Buffer and TE, pH 8.0. Mix by vortexing and spin down. Pipette 100 µL of the mixture on a 4–7 µm thick FFPE tissue on the microscope slide. Scrape the tissue off with a pipette tip and place the solution and tissue into a clean tube. Add 1.5 µL of DNARelease Additive.* Mix the solution with a pipette tip and spin down. Make sure the sample is covered with the solution. Incubate for 1 hour at 60 °C followed by 10 minutes at 98 °C. After cooling and centrifuging (16 000 x g, 2 min), transfer the supernatant into a new tube. Use 0.5 µL of the supernatant as a template in a 20 µL PCR reaction.

   In some cases, if the amount of tissue debris or DNA in the supernatant is very high, the supernatant may need to be diluted. Make a 1:10 or 1:100 dilution in $H_2O$ or TE buffer and use 0.5 µL of the dilution as a template in a 20 µL PCR reaction. **Note:** The DNA in FFPE tissue is usually fragmented, limiting the size of PCR products that can be successfully amplified. We recommend amplicon sizes less than 300 bp. In some cases extending the first incubation over night improves the PCR yield. *The protocol is optimized for 1–4 $cm^2$ tissue sections. For smaller or larger samples, adjust the volumes accordingly

### 6.2 Liquid samples

**Amniotic fluid**

1. Direct protocol: Add 0.5–2 µL of amniotic fluid directly into a 50 µL PCR reaction.
2. Dilution & Storage protocol: Not recommended

**Saliva**

1. **Direct protocol:** Add 0.2–0.5 µL of saliva directly into a 50 µL PCR reaction
2. **Dilution & storage protocol:** Add 5 µL of saliva in a tube that contains 20 µL Dilution Buffer and 0.5 µL DNARelease Additive. Mix by vortexing and spin down. Incubate at 98 °C for 2 minutes. Spin down again, and use 0.5 µL of the supernatant as a template in a 20 µL PCR reaction. **Note:** Only fresh saliva is recommended. If the saliva sample is to be stored for a longer period before PCR, it is recommended to use commercial saliva collection tubes (such as Oragene® DNA from Genotek).

## 7. Gel electrophoresis

2x Phire Plant Direct PCR Master Mix contains a premixed gel loading dye. After PCR samples can be directly loaded on the electrophoresis gel for analysis. It is recommended to use the DNARelease™ Additive for gel electrophoresis as otherwise cell debris present in the PCR products can cause DNA fragments to get trapped in the agarose gel wells. Add 1.5 µL of DNARelease Additive into a 50 µL PCR reaction.

## 8. Notes About Reaction Components

### 8.1 Phire Tissue Direct PCR Master Mix

2X Phire Tissue Direct PCR Master Mix has been optimized for Direct PCR from variety of tissues. It contains the dNTPs and provides 1.5 mM $MgCl_2$ concentration in the final reaction. It also includes a density reagent and two tracking dyes for direct loading of PCR product on a gel. The Master mix employes Phire Hot Start II DNA Polymerase, that 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.

### 8.2 Dilution Buffer

The Dilution Buffer has been optimized to release DNA from a wide variety of different tissues when supplemented with DNA Release Additive (see Section 8.3). Samples in Dilution Buffer can be stored for up to 4 weeks in different temperatures (-20 °C, +4 °C and room temperature) before using in PCR. For long term storage, it is recommended to transfer the supernatant into a new tube and store at –20 °C.

### 8.3 DNARelease Additive

DNARelease Additive is required 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 & Storage protocol to improve the release of DNA from the tissue sample.

### 8.5 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/tmc to determine the Tm values of primers and optimal annealing temperature.

## 9. Notes about cycling conditions

### 9.1 Initial denaturation

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

### 9.2 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.

### 9.3 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).

### 9.4 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.

## 10. Control reactions

### 10.1 Direct PCR control reaction using the control primer mix

When using mammalian tissue samples (e.g. mouse, human tissue), we recommend performing Direct PCR control reactions with both Direct and Dilution & Storage protocols using the control primers supplied with this Master Mix. 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 gene1 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 rxn | 50 µL rxn* | Final conc. |
|---|---|---|---|
| $H_2O$ | add to 20 µL | add to 50 µL | - |
| 2X Phire Tissue Direct PCR Master Mix | 10 µL | 25 µL | 1X |
| Universal control primer mix | 0.4 µL | 1 µL | 0.5 µM |
| Sample/Direct protocol: | – | Amount depends on the sample** | – |
| Sample/Dilution & Storage protocol: | 1 µL | 2.5 µL | – |

*50 µL reaction volume is recommended for the direct protocol.
**0.5 mm punch or a small sample of tissue(see www.

thermoscientific.com/directpcr)

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

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

## 10. References

1. Woolfe A. *et al.*(2005) PLoS Biology3: 116–130.

## 11. Shipping and storage

Upon arrival, store the components at –20 °C. The Dilution Buffer can also be stored at +4 °C once it is thawed.

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## 12. Troubleshooting

| | |
|---|---|
| **No product at all or low yield** | |
| Inhibition (positive control works with custom and control primers from the kit) | **Direct Protocol:**<br>Try to titrate sample amount. If not successful, try Dilution & Storage protocol. |
| | **Dilution & Storage Protocol:**<br>1:100 with H2O or TE buffer, and use 1 µL as a template in PCR. |
| Not enough template | **Direct Protocol:**<br>Make sure to perform the 2-minute incubation at 98°C.<br>Try incubating sample in Dilution Buffer at elevated temperature (up to 65°C) instead of room temperature. |
| | **Dilution & Storage Protocol:**<br>Try to titrate sample amount. |
| Problems with primer design | Positive control with purified DNA and custom primers fails, whereas there is product with control primers.<br>Assess your primer design. |
| Incorrect cycling parameters | Unoptimized threshold setting. |
| Incorrect annealing temperature | Optimize annealing temperature (run a temperature gradient). The optimal annealing temperature for Phire DNA Polymerase may differ significantly from that of Taq-based polymerases. Always use the Tm calculator and instructions on our website: www.thermoscientific.com/pcrwebtools. |
| Too short extension time | The recommended extension time is 20 seconds for amplicons ≤1 kb, and 20 s/kb for amplicons >1 kb. |
| Too less cycles | For difficult amplicons try using 40 cycles. |
| Samples trapped in wells | Make sure to add DNARelease Additive prior gel electrophoresis. |
| **Non-specific products** | |
| Problems with primers | Decrease primer concentration to 0.3 µM.<br>Design new primers.<br>Make sure these are not primer dimmers (when short amplicons are amplified). |
| Too low annealing temperature | Increase the annealing temperature or perform temperature gradient. |
| Adjust cycling times | Reduce the total number of cycles.<br>Shorten extension time. |
| Some DNA gets traped in wells | Add DNARelease Additive prior loading on gel. |

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