Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific Phire Plant Direct PCR Master Mix

#F-160S **100 rxns**

Lot 00000000 **Expiry Date ____**

Store at -20°C

Extended version of product information is available online www.thermoscientific.com/directpcr

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www.thermoscientific.com/onebio

COMPONENTS OF THE PRODUCT

Component	#F-160S 250 rxns x 20 µL 100 rxns x 50 µL	#F-160L 1250 rxns x 20 µL 500 rxns x 50 µL
2X Phire Plant Direct PCR Master Mix	2 × 1.25 mL	10 × 1.25 mL
Dilution Buffer	5 mL	2 × 12.5 mL
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 appl. (50 µg)	

Storage

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

Rev.5

1. Introduction

Thermo Scientific™ Phire™ Plant Direct PCR Master Mix is designed to perform PCR directly from different plant material without prior DNA purification: fresh plant leaves and seeds, plant material stored at 4 °C or frozen, plant material stored on commercially available cards such as Whatman® 903 and FTA® Cards. A list of plant species tested with this Master Mix is available at www.thermoscientific.com/directpcr.

The Master Mix contains premixed gel loading dye, which allows direct loading of PCR products 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.

2. Important Notes

- A more detailed protocol available at **www.thermoscientific.com/directpcr**
- Primer annealing temperatures for Phire are different from many common DNA polymerases (such as *Taq* DNA polymerases). Read Section 6.2 carefully.
- Use the Dilution & Storage protocol for difficult samples, long amplicons, and when you need multiple PCR reactions from the same sample.
- Samples in Dilution Buffer can be stored for up to 8 weeks at different temperatures (-20 °C, 4 °C and room temperature) before using in PCR.

3. Guidelines for PCR

Before starting read all Important notes (Section 2) and Sample handling guidelines (Section 4). Thaw, mix and spin down all solutions, keep on ice. The PCR setup can be performed at room temperature.

Table 1. Pipetting instructions

Component	20 µL rxn	50 µL rxn	Final conc.
2X Phire Plant 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
Plant tissue **Direct Protocol** **Dilution & Storage Protocol**	- 0.5 µL	0.5 mm punch/ sample of seed 1.25 µL	
H_2O	add to 20 µL	add to 50 µL	

*50 µL reaction volume is recommended for Direct Protocol.

Table 2. Recommended cycling protocol

Cycle step	2-step		3-step		Cycles
	Temp.	Time	Temp.	Time	
Initial denaturation	98 °C	5 min	98 °C	5 min	1
Denaturation Annealing (see 6.2) Extension (see 6.3)	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	35 - 40
Final Extension	72 °C 4 °C	1 min hold	72 °C 4 °C	1 min hold	1

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.

Positive control reaction with purified DNA

When optimizing the direct PCR protocol, 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 until the control PCR gives a desired result.

Negative control

It is recommended to use a no-template control with all direct PCR assays to control contamination. To monitor the efficiency of cleaning the sampling tool, 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.

4. Guidelines for Sample Handling

To obtain small and uniform samples, we recommend using 0.35 – 0.50 mm diameter puncher. If the puncher is to be reused, it is very important to clean the cutting edge properly to prevent cross-contamination between samples. Use 2% NaClO solution for cleaning and cross contamination prevention.

Other ways to take a sample is by cutting with scalpel to obtain 0.35 – 0.50 mm sample. Scalpel must be cleaned properly to prevent cross-contamination between samples.

4.1. Plant leaves

Direct protocol: Take a sample from the plant of approximately this size (•), corresponds to 0.5 mm punch disc. Place the disc directly into the PCR reaction (50 µL volume). It is recommended to eject the disc into a liquid, rather than onto the wall of an empty tube. Make sure that you see the sample disc in the solution. We recommend using young leaves. Fresh plant material is the best choice, however plant material stored at 4 °C, frozen, or on cards such as Whatman 903 or FTA can also be used (see section 4.3). For long fragments or difficult samples with direct protocol, a smaller 0.35 mm punch disc may give more robust results.

Dilution & Storage protocol: As with the direct protocol, young leaves are recommended. Take one small leaf or a piece of a leaf (e.g. a punch approximately 2 mm in diameter) and place it in 20 µL of Dilution Buffer. Crush the leaf sample with a pipette tip by pressing it briefly against the tube wall. If larger amount of leaf tissue is used (do not exceed 1 mg), increase the volume of the Dilution Buffer to 50 µL. After crushing the leaf, the solution should be greenish. Spin the plant material down, and use 0.5 µL of the supernatant as a template in a 20 µL PCR reaction. The required volume of the supernatant may vary depending on the plant material used and the volume used for the dilution.

4.2. Plant seeds

Direct protocol: Using a clean scalpel, remove the seed coat and cut a small sample of the seed (approximately the size of this dot •). Place the sample directly into the PCR reaction (50 µL in volume). Note that it is recommended to use dehulled seeds. For very small seeds (such as *Arabidopsis*), use 1−2 whole seeds and place them directly into the PCR reaction.

Dilution & Storage protocol: Cut a small sample of the dehulled seed by using a scalpel (approximately the size of this dot (•) and place it directly into 20 µL of Dilution Buffer. Briefly vortex the tube and incubate at room temperature for 3 min. Make sure that the seed sample is covered with Dilution Buffer. Spin briefly and use 0.5 µL of the supernatant as a template for a 20 µL PCR reaction.

4.3. Plant material stored on commercially available storage cards, e.g. Whatman 903 and FTA Cards

Direct protocol: Take 0.5 mm punch disc from the sample on the storage card. Place the disc directly into a 50 µL PCR reaction. For amplifying long fragments or difficult samples, a 0.35 mm punch disc may give more robust results.

5. Notes about reaction components

5.1. 2X Phire Plant Direct PCR Master Mix

2X Phire Plant Direct PCR Master Mix has been optimized for Direct PCR from variety of plant 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 employs 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 DNA 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 dA-overhangs to the blunt PCR product with Taq DNA Polymerase, (protocol available at www.thermoscientific.com/pcrcloning).

5.2. Dilution Buffer

The Dilution Buffer has been optimized to release DNA from a wide variety of different sample materials such as plant leaves and seeds. This buffer is also suitable for storing the DNA sample up to 8 weeks at +4 °C or room temperature. For long term storage keep at −20 °C. Before storage it is recommended to transfer the supernatant into a new tube.

6. Notes about cycling conditions

6.1. Initial denaturation

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

6.2. 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 at 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 annealing temperature equal to the Tm of the lower Tm primer. In some cases, it is beneficial 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).

6.3 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

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 plant material as in the actual experiment. If the PCR using control primer mix is not working, the plant sample may not be suitable for direct PCR. Control primers are supplied as a mix of primers in H_2O that amplify a 297 bp fragment of a highly conserved region of chloroplast DNA[1]. The control primer mix has been validated with a large number of species (refer to the list of tested plants at www.thermoscientific.com/directpcr). Each primer concentration is 25 µM.

Primer #1 (20-mer)
5'- AGTTCGAGCCTGATTATCCC -3'
Melting point: 62.4 °C
Primer #2 (20-mer)
5'- GCATGCCGCCAGCGTTCATC -3'
Melting point: 75.5 °C

Table 3. Pipetting instructions for control reactions.

Component	20 µL rxn
2X Phire Plant Direct PCR Master Mix	10 µL
Control primer mix	0.4 µL
Plant tissue **Direct protocol**	0.35 mm punch/ sample of seed
Dilution & Storage protocol	0.5 µL supernatant
H_2O	add to 20 µL

Table 4. Cycling instructions for control reactions.

Cycle step	Temp.	Time	Cycles
Initial denaturation	98 °C	5 min	1
Denaturation Annealing Extension	98 °C 62 °C 72 °C	5 s 5 s 20 s	40
Final Extension	72 °C +4 °C	1 min hold	1

CERTIFICATE OF ANALYSIS

Performance in PCR is tested by the amplification of 7.5 kb fragment from human genomic DNA. Absorption measured at 424 nm and 614 nm.

Quality authorized by:  Jurgita Zilinskiene

REFERENCES

1. Demesure B. *et al.* (1995) *Molecular Ecology* 4: 129–131.

TROUBLESHOOTING

To optimize Direct PCR three key steps have to be considered: dilution protocol, sample size and optimal primer annealing temperature.

Troubleshooting information is available in the extended version of the protocol. See www.thermoscientific.com/directpcr for more details.