Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific Phire Plant Direct PCR Kit

#F-130WH **200 rxns**

Lot __ **Expiry Date __**

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www.thermoscientific.com/onebio

Ordering information

Component	#F-130WH 500 rxns × 20 µL 200 rxns × 50 µL
Phire Hot Start II DNA Polymerase	200 µL
2X Phire Plant PCR Buffer (includes dNTPs and $MgCl_2$)	5 × 1.25 mL
Control Primer Mix (25 µM each)	40 µL
Dilution Buffer	2 × 5 mL

Storage

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

CERTIFICATE OF ANALYSIS

Performance in PCR is tested by the amplification of 7.5 kb fragment from human genomic DNA.

Quality authorized by: Jurgita Zilinskiene

Rev.4 53

1. Introduction

Thermo Scientific™ Phire™ Plant Direct PCR Kit is designed to perform PCR directly from plant leaves and seeds without prior DNA purification. Fresh plants, plant material stored at 4 °C or frozen are all suitable templates for this kit, as well as plant material stored on commercially available cards such as Whatman® 903 and FTA® Cards. A list of plants tested with this kit is available at www.thermoscientific.com/directpcr. The 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 plants. The Phire Plant Direct PCR Kit contains reagents and tools for two alternative methods: direct and dilution protocols. Dilution Buffer is included for optional sample treatment before PCR (see 'Dilution protocol' in Section 4). It can be used to treat larger/more difficult samples (e.g. more fibrous or latex-containing samples), or when multiple PCR reactions are performed from a single sample. It is also a useful choice when longer DNA fragments (> 1 kb) are amplified. The kit includes control primers for amplification of a highly conserved region of chloroplast DNA[1]. The kit is recommended for end-point PCR protocols.

2. Important Notes

- Clean the sampling tools between each sample.
- Add the sample into a PCR reaction instead of an empty tube.
- Use the dilution protocol for difficult samples, for long amplicons or for performing multiple reactions from the same sample.
- Use 98 °C for denaturation.
- The annealing temperatures for Phire are different from many common DNA polymerases (such as *Taq* DNA polymerases). Read Section 6.2 carefully.
- For extension, use 20 s for amplicons <1 kb or 20 s/kb for amplicons > 1 kb.

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.

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 Plant 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	
Plant tissue (see Section 4) Direct protocol Dilution protocol	0.5 mm diameter leaf sample/small sample of seed 0.5 µL	0.5 mm diameter leaf sample /small sample of seed 1.25 µL	

Table 2. Recommended 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 6.2) 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	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 a puncher that is 0.5 mm or 0.35 mm in diameter. 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% NaOCl 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 0.5 mm diameter sample from the plant leaf and place it directly into the PCR reaction (20−50 µL in volume). It is recommended to add sample into a liquid, rather than onto the wall of an empty tube. Make sure that you see the sample in the solution. We recommend using young leaves. Fresh plant material is usually the best choice, even though plant material stored at 4 °C, frozen or on commercially available cards such as Whatman 903 and FTA Cards can also be used (see section 4.3). For amplifying long fragments or difficult samples using the direct protocol, a smaller sample (e.g. a punch of 0.35 mm in diameter) may give more robust results.

Dilution protocol: As with the direct protocol, young leaves are recommended. Take one small leaf or a piece of 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 100 µL 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 in colour. Spin the plant material down, and use 0.5 µL of the supernatant as a template for 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 (20−50 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 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: Use the 0.50 mm diameter sample from the storage card. Place the sample directly into a 50 µL PCR reaction. For amplifying long fragments or difficult samples, a smaller sample of 0.35 mm diameter may give more robust results.

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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. Phire Hot Start II DNA Polymerase produces blunt ends and therefore blunt end cloning is recommended. If TA cloning is required, it can be performed by adding A overhangs to the blunt PCR product with *Taq* DNA Polymerase, for example (protocol available at www.thermoscientific.com/pcrcloning).

5.2. Phire Plant PCR Buffer

The 2X Phire Plant PCR Buffer has been optimized for direct PCR from plant material. 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 sample materials such as plant leaves and seeds. This buffer is also suitable for storing the DNA sample for 8 weeks at 4 °C. For long term storage, it is recommended to transfer the supernatant into a new tube and store at −20 °C. The Dilution Buffer is sufficient for 500 dilution reactions 20 µL each.

5.4. Primers

The recommendation for 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 at 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. 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 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).

6.3 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 protocols using the control primers supplied with this kit. 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. 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	Final conc.
H_2O	add to 20 µL	
2X Phire Plant PCR Buffer	10 µL	1X
Control primer mix	0.4 µL	0.5 µM
Phire Hot Start II DNA Polymerase	0.4 µL	
Plant tissue (see Section 4) Direct protocol:	0.35 mm diameter leaf sample/small sample of seed	
Dilution protocol	0.5 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

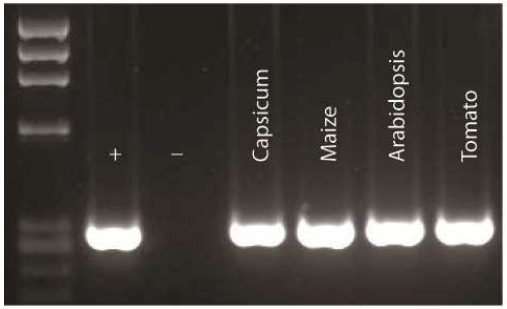

Figure 1. Amplification of the 297 bp control DNA fragment directly from Capsicum, maize, Arabidopsis and tomato leaves. A 0.50 mm puncher was used to cut a sample disc from the plant leaves. The samples were placed directly into 20 µL PCR reactions. After PCR, 5 µL of loading buffer was added to the reaction and 15 µL was used for gel electrophoresis. + denotes the control reaction with purified plant DNA and – is 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 (see Section 8).

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 puncher, the cleaned sampling tool can be dipped into the negative control sample. A second negative control performed without dipping the puncher is recommended to control other sources of contamination.

References

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

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8. Troubleshooting

No product at all or low yield

General
If the positive control with purified DNA using your own primers is not working:

- Optimize annealing temperature.
- Make sure the cycling protocol was performed as recommended.
- Check primer design.

Direct protocol
If the positive control with purified DNA and the Direct PCR control reaction with control primers are working, but the actual samples yield no product:

- Increase the PCR reaction volume to 50 µL or use a smaller sample (e.g. 0.35 mm punch).
- Increase the number of cycles.
- Use dilution protocol for amplification of large or difficult samples and long DNA fragments.

Dilution protocol
If the positive control with purified DNA and the Direct PCR control reaction with control primers are working, but the actual samples yield no product:

- Dilute the supernatant (from the sample treated with Dilution Buffer) 1:10 and/or 1:100 with H_2O/TE buffer, and use 0.5 µL as a template in PCR.
- Try both crushing and not crushing the sample.
- Incubate the sample in Dilution Buffer for 3 min at room temperature and use 0.5 µL as a template (1:1 and 1:10 dilution) in a 20 µL reaction.
- Use smaller sample size or increase the volume of Dilution Buffer.

Non-specific products - High molecular weight smears

- Make sure the extension time used was not too long (>20 s/kb).
- Reduce the total number of cycles.
- Increase annealing temperature or perform a temperature gradient PCR.
- Decrease primer concentration.
- Make sure the cutting tools were properly cleaned with 2% sodium hypochlorite. Include a negative control.

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.
- Make sure the cutting tools were properly cleaned with 2% sodium hypochlorite. Include a negative control.

PRODUCT USE LIMITATION
This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.

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