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Plant directPCR 2x Master Mix

♯3202
Store at -20°C

Contents

Plant directPCR 2x Master Mix contains all the components for directPCR from plants, including an engineered DNA polymerase, a specifically developed buffer system, and ultrapure dNTPs. A hot-start formulation of the included DNA polymerase suppresses false amplification. A specifically developed lysis buffer and control primer mixes for plant DNA are also included in the kit.

Description

DNA isolation is not needed anymore - Plant directPCR 2x Master Mix allows the PCR directly from leave material after a quick lysis step.
The included DNA polymerase was engineered to display a very high resistance against many types of common PCR inhibitors, as well as for robustness and selectivity. It comes together with an optimized buffer system and a separate lysis buffer.
Plant directPCR 2x Master Mix ensures reproducible results, significantly reduces set-up times and the risk of pipetting mistakes. It can also be used for real-time cycling when adding a suitable real-time dye.

Applications

- Direct PCR from plants (fresh, stored at 4°C or frozen)
- Direct gene detection and quantification
- Standard PCR
- Real-time PCR
- Screening / High-throughput PCRs

Recommendations for PCR

PCR Mix

Component	Volume	Final concentration
Plant directPCR 2x Master Mix	12,5 µl	1x
Primer forward (10 µM)*	0.25 µl	0.2 µM (0.05-1 µM)
Primer reverse (10 µM)*	0.25 µl	0.2 µM (0.05-1 µM)
Template/Sample extract	5 µl	according to reaction setup
Nuclease-free water	up to 25µl total vol.	

*Primers should ideally have a GC content of 40-60%

Typical 3-step PCR protocol

Initial denaturation	95°C	3 min	
Denaturation	95°C	10 sec	25-40cycles
Annealing*	54–72°C	60sec/1000bp	
Hold	<10°C		

*For a new PCR a temperature gradient to find the optimal temperature is recommended.

Reaction setup/ template preparation

For most fresh plants we recommend, that a 1-4 mm^2 part of the leave[2] is diluted in 50 µl of the provided lysis buffer and vortexed for 30 sec to 1 minute at room temperature. Samples can either be cut with a scalpel or with a puncher. To prevent cross-contamination between samples the scalpel/ puncher must be cleaned properly after each sample.
After the lysis step the lysate should have a light green colour but not be intensively green.
Alternatively, if a centrifuge is available, a 1-4 mm^2 part of the leave[2] can be diluted in 50 µl of the provided lysis buffer by gently pressing or rubbing the leave against the tube. Before proceeding with the PCR set-up, the lysate should be centrifuged for 1 minute by 10.000 rpm.

[2] approximately the size of this square (4mm^2)

The so prepared lysate can be stored on ice for at least one hour. As template 5 µl[3] of the supernatant is directly used for a 25 µl PCR reaction. Please note that the amount of lysate derived from one plant sample is sufficient for approximately 5 to 8 PCR reactions. For more reactions from one single plant sample we recommend to scale the lysate reaction up according to your needs.

[3] the optimal quantity of supernatant might depend on the plant species.

Real-time PCR
Plant directPCR 2x Master Mix can also be used for real-time PCR, when adding a suitable real-time dye. Please note that high concentrations of sample extracts might quench the fluorescence signal.

Control reactions for plant DNA
Primer control mix for plant DNA is included in the kit, as a 10x ready to use (1 µM each) mix. For the control reaction we recommend an annealing temperature of 63°C and an extension time of 60 sec.
The plant specific control primer mix included in the kit amplifies a 297 bp fragment of a highly conserved region of chloroplast DNA. The primer mix can be used with a large number of plant species. Primer sequences are (also see reference 1):

Primer Plant 1 (20 nt) : 5'-AGTTCGAGCCTGATTATCCC -3'
Primer Plant 2 (20 nt) : 5'-GCATGCCGCCAGCGTTCATC -3'

Recommendations for sample handling

- Keep all components on ice.
- Spin down and mix all solutions carefully before use.
- Primers should ideally have a GC content of 40-60%.
- Minimize the number of freeze-thaw cycles by storing in aliquots. For a day-to-day use, we recommend keeping an aliquot at 4°C.

Quality Control Assays
PCR activity: Plant directPCR 2x Master Mix was tested for successful PCR performance. A 297bp fragment (chloroplast)[1] was amplified from a plant leave and analysed by agarose gel electrophoresis.
DNA polymerase activity: DNA polymerase activity has been monitored and adjusted to a specific DNA polymerase activity using an artificial DNA template and a DNA primer.
Enzyme-concentration has been determined by protein-specific staining. Please inquire more information at info@mypols.de for the lot-specific concentration.
No contamination has been detected in standard test reactions.

Storage
This product is shipped on cool packs. Please store the product upon arrival at -20°C. Minimize the number of freeze-thaw cycles by storing in aliquots. For a day-to-day use, we recommend keeping an aliquot at 4°C.

Safety
This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally recommend the use of gloves, lab coats and eye protection when working with these or any other chemical reagents. myPOLSBiotec takes no liability for damage resulting from handling or contact with this product. Further information can be found in the REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL.

References

1. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Mol. Ecol. 1995; 4: 129–131. B. Demesure, N. Sodzi, and R. J. Petit