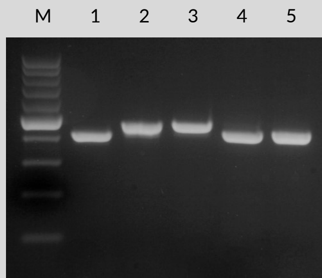
EURx® MOLECULAR BIOLOGY PRODUCTS

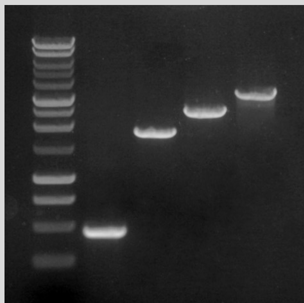
Direct Plant PCR Kit

Cat. No.	size
E0960-01	100 reactions of 50 µl
E0960-02	500 reactions of 50 µl

Storage Conditions: Store at -20°C.
For short term storage (up to 2 weeks) Extraction Solution and Dilution Solution may be kept at 4°C.



PCR amplification from different plant tissues and species using the Direct protocol of EURx Direct Plant PCR Kit.
Conserved PCR primers were used for amplification of chloroplast DNA (ca 400 bp fragment).
Lane M: molecular size marker – Perfect Plus 1 kb DNA Ladder (E3131).
Lanes 1 to 5: PCR amplification reactions directly from (in order): grass leaf, parsley leaf, apple seed, rubber plant leaf, spruce neadle.



PCR amplification from parsley leaf using the Extract protocol of EURx Direct Plant PCR Kit.
Conserved PCR primers were used for amplification of chloroplast DNA.
Lane M: molecular size marker – Perfect Plus 1 kb DNA Ladder (E3131).
Lanes 1 to 4: PCR amplification reactions (fragments from 0.4 up to 3.5 kb) directly from parsley leaf using the Extract protocol of EURx Direct Plant PCR Kit.

Direct Plant PCR Kit enables to perform PCR reactions directly from plant samples with no prior DNA purification.

Description:

- The Direct Plant PCR Kit allows to use samples such as: plant leaves , plant seeds and plant material stored on commercially available cards.
- The samples can either be fresh or stored at -20°C.
- The Direct Plant PCR Kit employs a hot start and genetically engineered thermophilic DNA polymerase with high tolerance to plant inhibitors.
- DNA Polymerase activity is restored during 10 min initial denaturation step.
- DNA Polymerase catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction.
- The enzyme exhibits the 3'→5' proofreading activity, resulting in 50-fold higher PCR fidelity than possible with Taq DNA Polymerases.
- The enzyme generates blunt ends.
- Enhanced polymerase processivity allows to use shorter extension times.
- **Due to the genetic modification of the polymerase, the optimal reaction conditions (especially annealing temperatures) differ from standard PCR protocols**.
- The Direct Plant PCR Kit contains reagents for two alternative protocols: Direct and Extract protocols.
- The master mix contains premixed gel loading reagent and dyes which allow direct sample loading on the gel.
- The Direct Plant PCR Kit allows to obtain a wide range of product size (over 4 kb).

Direct Plant PCR Kit contains:

1. 2 x Plant PCR Master Mix
2. DNA Polymerase
3. Extraction Solution
4. Dilution Solution
5. Water, nuclease free

2 x Plant PCR Master Mix:

The master mix contains 2 x concentrated optimized PCR buffer, 5 mM $MgCl_2$, dNTPs and two gel tracking dyes.

DNA Polymerase Storage Buffer:

20 mM Tris-HCl (pH 9.0 at 22°C), 100 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 50% glycerol and stabilizers.

Quality Control:

All preparations are assayed for contaminating 3'-exonuclease, and nonspecific single - and double-stranded DNase activities. Typical preparations are greater than 95% pure, as judged by SDS polyacrylamide gel electrophoresis.

This product is developed, designed and sold exclusively for research purposes and in vitro use only.

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Sample handling

To obtain small and uniform samples, we recommend using 0.35-0.5 mm diameter puncher or scalpel. If the puncher or scalpel is to be reused, it is important to clean the cutting edge properly to prevent cross-contamination between samples. Use 2% NaClO solution for cleaning and cross-contamination prevention.

Choosing the protocol

The Direct Plant PCR Kit contains reagents for two alternative protocols: Direct and Extract protocols. With a few exceptions, both Direct and Extract protocol are compatible with all sample types and application.

The Extract protocol is recommended:

- With difficult or long amplicons (over 1 kb).
- When performing multiple reactions from the same sample.

For Extract protocol use 20-50 µl reaction volume and for Direct protocol use only 50 µl reaction volume.

Direct protocol

Take a sample of 0.35-0.5 mm in diameter from plant leaves, seeds or plant material stored on commercially available cards.

It is recommended to use young leaves or dehulled seeds. For very small seeds, use 1-2 whole seeds.

Place the sample directly into a 50 µl PCR reaction. Perform PCR (see the table on the next page).

For difficult samples (rich in polyphenols), a smaller 0.35 mm punch may give more robust results.

Extract protocol

Place the plant sample of 0.35-1 mm in diameter into 50 µl of Extraction Solution. Incubate the reaction for 5 minutes at room temperature. Add 50 µl of Dilution Solution, mix the tube briefly and spin down the solution. Store the supernatant at 4°C or –20°C. The samples in mix of Extraction and Dilution Solutions can be stored for up to 8 weeks before using in PCR. For long term storage keep at -20°C. Use 1 µl of the supernatant as a template in a 20 µl PCR reaction.

Preparation of PCR Reaction From Plant Sample:

Component	20 µl reaction	50 µl reaction	Final concentration
2 x Plant PCR Master Mix	10 µl	25 µl	1x 2.5 mM $MgCl_2$
Primer A	Variable	Variable	0.5 µM
Primer B	Variable	Variable	0.5 µM
DNA Polymerase	0.4 µl	1 µl	
Sample Direct protocol Extract protocol	 - 1 µl	 0.35-0.5 mm plant sample 2.5 µl	
Sterile double-distilled water	Add to 20 µl	Add to 50 µl	
Total volume	20 µl	50 µl	

Notes:

1. Completely thaw and mix thoroughly all components of PCR reaction before use to avoid localized differences in salt concentration.
2. Prepare reaction mixes at room temperature. Use of DNA Polymerase allows room temperature reaction setup. Mix well.
3. Reactions can be placed in a room temperature thermal cycler.
4. The 2 x Plant PCR Master Mix allows PCR reactions to be loaded directly onto an agarose gel without prior addition of a gel loading buffer. The master mix contains a gel loading reagent and two gel tracking dyes (a red dye and a yellow dye) that separate during electrophoresis. In a 1% agarose gel, the red dye migrates at the same rate as 600 bp DNA fragment and the yellow dye migrates faster than 20 bp. The dyes do not interfere with most downstream enzymatic applications, however it is recommended to purify PCR products prior enzymatic manipulation.
5. In most cases there is no need to add additives to PCR reaction. For some difficult targets such as: GC-rich sequences, sequences with complex secondary structures additives such as DMSO can be included to improve amplification. The recommended starting DMSO concentration is 3% (if needed).

Thermal Cycling Conditions:

Step	2-step protocol		3-step protocol		Number of Cycles
	Temp.	Time	Temp.	Time	
Lysis of Cells, Initial Denaturation	98°C	10 min	98°C	10 min	1
Denaturation	98°C	5-10 s	98°C	5-10 s	35-40
Annealing	-	-	X°C	15-30 s	
Extension	72°C	30 s/1 kb	72°C	30 s/1 kb	
Final Extension	72°C	1 min	72°C	1 min	1
Cooling	4°C	Indefinite	4°C	Indefinite	1

Notes:

1. A 10-minute initial denaturation at 98°C enables lysis of cells and DNA Polymerase activation.
2. DNA Polymerase has the ability to stabilize primer-template hybridization. Melting temperatures (T_m) and optimal annealing temperatures usually differ significantly (are higher) from the temperatures calculated/determined for standard PCR polymerases. T_m's should be calculated with the base-stacking method (nearest-neighbor method) using calculator on the website: http://eurx.com.pl/CALC/. Default parameters are: 500 nM primer concentration, 50 mM salt concentration, 1.5 mM Mg^{2+} concentration. As a basic rule use an annealing temperature at a T_m of the lower T_m primer. In some cases optimal annealing temperatures may differ from the rule given above and should be determined empirically.
3. A 2-step protocol allows to perform combined annealing/extension step at 72°C and is recommended for primers with T_m values of at least 72°C. The 2-step protocol allows to save time of PCR reaction.
4. Extension time of 30 s/ 1 kb is recommended for most targets.

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