FastAmp™ Plant Direct PCR Kit

Catalog #	Package Size	Concentration
4612	250 reactions	2x
4615	1,250 reactions	2x

Description

FastAmp™ plant direct PCR master mix is suitable for amplification of DNA directly from plant samples without purifying DNA. This kit is based on specially engineered *Taq* DNA polymerase, proprietary buffer system, dNTP, $MgCl_2$, PCR facilitators and dye mix which makes it extremely robust and tolerant of plant PCR inhibitors such as complex polysaccharides, polyphenols and others. This PCR master mix has been tested with leaves and seeds from a wide variety of plant species. This kit includes a complete set of optimized reagents and detailed protocols making it an ideal choice for amplification of plant DNA without DNA purification.

Highlights

- Direct PCR- no need to purify DNA
- Specially engineered *Taq* DNA polymerase with highest sensitivity and specificity
- Extremely short PCR protocol times
- Master mix format with premixed gel loading dye to reduce cross-contamination and sample handling errors
- 5x magic enhancer for high GC containing DNA amplification

Applications

- Genotyping
- Transgene detection
- Knockout analysis
- Sequencing

Product Includes

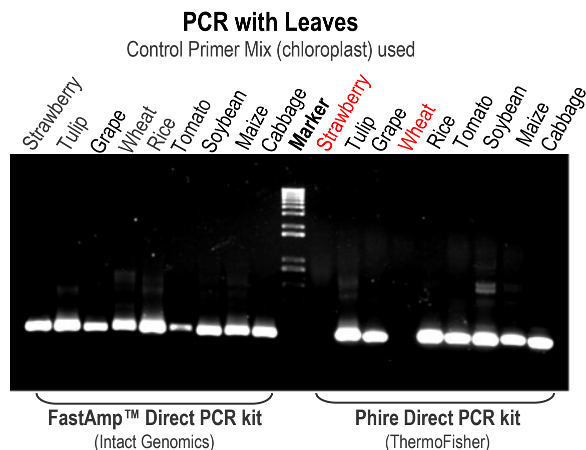
- FastAmp™ plant direct PCR master mix (2x)
- Dilution buffer
- Control primer mix (25 µM each)
- 5x magic enhancer
- Nuclease- free water

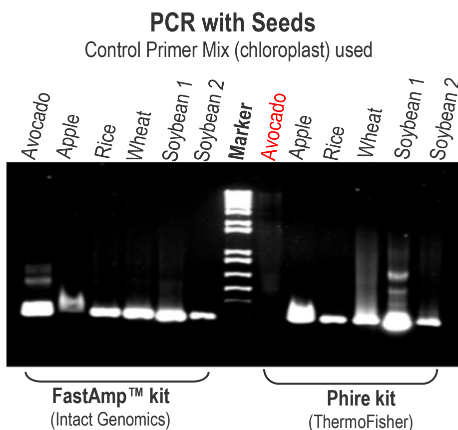
Storage Temperature

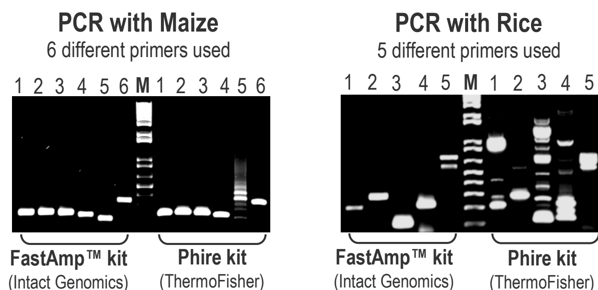
-20ºC

Quality Control Assays

FastAmp™ Plant Direct PCR Kit has been tested with leaves and seeds from a wide variety of plant species, some of the results are included here.







General Guidelines before start

A. Sample handling

A leaf punch (ø 0.5 to 1.2 mm) can be obtained by placing the leaf puncher (Uni-Core punch by GE Healthcare, Product # WB100028, or any other commercial sources) in perpendicular position over the expanded leaf and rotating it. The tip of puncher need to place inside PCR tube and expunge to drop the sample. It is critical that plant tissue materials/leaf punch is completely inside the PCR solution in tube. It is very likely that leaf punch sticks to the side of PCR tube and sometimes does not go at the bottom of tube, therefore, use a 10 µl pipet tip to manually drive the leaf punch to the bottom of PCR tube. This can be done right before capping the PCR tubes and starting the thermocycling program. For reusing the puncher, it is very important to clean the cutting edge properly with 70% ethanol to prevent cross-contamination between samples.

For seed amplification, first grind the seed and place it into 100 µl of proprietary dilution buffer. Briefly mix the tube and incubate at 95 ºC for 5-10 min. Spin down and use 1-2 µl of the supernatant as a template for a 20 µl PCR reaction.

B. Control reaction

It is recommended that universal control primers provided with this kit to be used as a positive control in all plant direct PCR reactions. Control primers are supplied as a mix of primers in H2O that amplify a 297 bp fragment of a highly conserved region of chloroplast DNA. Optimum annealing temperature for control primers is 58 ºC. As a template, use the same plant material as in the actual experiment. If the PCR using control primer mix does not work, then the plant sample may not be suitable for direct PCR. When optimizing the PCR reactions, it is recommended to perform a positive control with purified DNA to ensure that the PCR conditions are optimal.

C. PCR conditions

Denaturation:

An initial denaturation of 5 minutes at 95°C is sufficient for most amplicons. Longer denaturation times can be used (up to 8 minutes) for difficult templates.
During thermocycling, the denaturation step should be kept to a minimum. Typically, a 20–30 second denaturation at

95°C is recommended for most templates.

Annealing:
Optimize the annealing temperatures for the target gene specific amplification by keeping annealing temperature at least 5 ºC below Tm values. Typically, use a 10–30 second annealing step. A temperature gradient can also be used to optimize the annealing temperature for each primer pair.

Extension:
The recommended extension temperature is 72°C. Extension times are generally 1 minute per kb for complex, genomic samples, but can be reduced to 30 seconds per kb for simple templates. When amplifying products >2 kb, it is often helpful to increase the extension time.

A final extension of 5 minutes at 72°C is recommended.

Cycle number:
Generally, 35–40 cycles yield sufficient product.

Primers:
Forward and reverse primers are generally used at the final concentration of 0.1-0.6 μM each. Too high a primer concentration can reduce the specificity of priming, resulting in non-specific products.

PCR product:
The PCR products generated using Taq DNA Polymerase have dA ends. If cloning is the next step, then T/A-cloning is preferred.

Protocol

The reaction mix typically contains all the components needed for PCR except DNA template (leaf punch/other sources).

1. Thaw 2x master mix, primer solutions, 5x magic enhancer (if required), mix thoroughly and spin down before use.
2. Prepare a reaction mix according to the following table:

PCR Reaction Set Up:	
Leaf punch	0.5 to 1.2 mm
FastAmp™ plant direct PCR master mix (2x)	10.0 μl
Forward primer (3.2 μM)	1 μl
Reverse primer (3.2 μM)	1 μl
5x Magic enhancer (optional)	*(4 μl)*
H_2O up to	20.0 μl

3. Mix the reaction mixture thoroughly.
4. Add leaf punch at the bottom of the individual PCR tube containing the reaction mixture.
5. Program the thermal cycler according to the manufacturer's instructions. A typical PCR cycling program is outlined in the following table.
6. Place the PCR tubes in the thermal cycler and start the cycling program.

PCR Cycling Conditions:			
Steps	**Temp.**	**Time**	**Cycles**
Initial denaturation	95 ºC	5 min	1
Denaturation	95 ºC	20-30 sec	35-40
Annealing	Tm-5 ºC	20-30 sec	
Extension	72 ºC	1 mim / kb	
Final extension	72 ºC	5-10 min	1
Hold	4-12 ºC	∞	

7. Run 10.0 μl of PCR products in 1% agarose gel (140 volts for 45 min).

Troubleshooting

No product at all or low yield
If the positive control with purified DNA is not working: • Optimize annealing temperature • Make sure the cycling protocol was performed as recommended • Increase the number of cycles up to 40 • Use dilution buffer and follow the seed protocol for amplification of large or difficult samples and long DNA fragments.

Non-specific products
• Increase annealing temperature • Shorten extension time • Decrease primer concentration • Check the purity and concentration of primers • Re-design new primers and test several pairs of primer