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# AmpEasy™ Plant Direct PCR Kit

## Description

AmpEasy™ Plant Direct PCR Kit is designed for performing PCR directly from plant leaf tissues without DNA purification or sample preparation. AmpEasy™ Plant Direct PCR Kit has been validated for the direct amplification of DNA fragments from leaves and seeds from a wide variety of plant species. Fresh plants, plant material stored at +4℃ or frozen at -20℃ ar e all suitable templates for this kit, as well as plant material stored on commercially available cards such as Whatman 903 and FTA Cards.

AmpEasy™ Plant Direct PCR Kit is optimized to give excellent results with plant leaves originated from a wide variety of plant species. AmpEasy™ Plant Direct PCR Kit also exhibits extremely high resistance to many PCR inhibitors found in plants, such as polyphenolics and polysaccharides. AmpEasy™ Plant Direct PCR Kit gives excellent results with plant leaves even with high polyphenolic compounds. AmpEasy™ Plant Direct PCR Kit includes a complete set of optimized reagents, sampling tools required for plant PCR except the template (plant tissue) and primers. By using AmpEasy™ Plant Direct PCR Kit, DNA fragments may be amplified directly from reactions containing 0.5-1mm diameter of plant tissue without pretreatment of plant tissue samples or DNA isolation, significantly reducing contamination risk, turnaround time and cost of genetic testing.

## Features

- PCR can be performed directly from plant tissue without DNA purification or sample preparation.
- Direct amplification of plant tissue samples stored at +4℃, frozen at -20℃ and stored on cards.
- No DNA purification is required, minimizing pipetting tasks and saving plenty of time.
- All components required for plant PCR are supplied except the template (plant tissue) and primers.

## Applications

- Ideal for analysis of large numbers of different plant samples
- Multiplex PCR / SNP detection / PCR-RFLP / Quantitative PCR
- Genotyping / Transgene detection

## Quality Control

Sensitivity and reproducibility of AmpEasy™ Plant Direct PCR Kits are tested in reproducibility assay: parallel 25µl reactions containing 0.5-1mm diameter of plant tissue and 1 µl of plant control primer mix. After 40 cycles, PCR products are verified by electrophoresis and DNA sequencing.


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## Shipping and Storage Conditions

AmpEasy™ Plant Direct PCR Kit is shipped on dry ice and should be stored immediately upon receipt at -20℃ in a constant temperature freezer. With prope r storage, AmpEasy™ Plant Direct PCR Kit can be stored for up to 12 months without showing any deduction in performance and quality.

## Product Components and Storage Conditions

| Cat. No.: | PDP100 |
|---|---|
| Product Name: | AmpEasy™ Plant Direct PCR Kit |
| Size (25 µl/reaction): | 100 preps |
| AmpEasy™ Plant DNA Polymerase | 100 ul |
| 2X AmpEasy™ Plant PCR Buffer* | 1.25 ml |
| Plant Control Primer Mix (5 µM each)# | 100 ul |
| $MgSO_4$ Solution | 200 ul |
| EDTA Solution | 200 ul |
| Harris Uni-Core puncher (0.5 mm) | 1 unit |
| Cutting Mat | 1 unit |

* Includes $Mg^{2+}$ and dNTPs.

# Contains plant control forward primer and plant control reverse primer (Size: 236 bp; Tm : 55℃)

# Protocol

## Important Notes Before Starting:

1. To prevent cross-contamination between samples, it's very important to clean the cutting tool (ex: the Harris Uni-Core puncher) between each sample by dipping it into either 70% EtOH or 2% bleach solution and press the plunger up and down at least 5 times. After cleaning, wipe the tip with a clean paper towel. The cutting mat must be rinsed with the same solution after each sampling.
2. Do not add $Mg^{2+}$ and dNTPs to the PCR reaction since they are already included in 2X AmpEasy™ Plant PCR Buffer.
3. For high PCR inhibitors in plants, optimization of $Mg^{2+}$ concentration may be required. $MgSO_4$ Solution and EDTA Solution are also provided for optimization of $Mg^{2+}$ concentration. For a 25ul reaction, adding 1ul of $MgSO_4$ Solution is equivalent to 1mM $MgSO_4$ in final concentration. Please note that excess $Mg^{2+}$ may result in spurious PCR products. Also if unspecific products are created, the effective $Mg^{2+}$ concentration can be decreased by adding the chelating agent EDTA. Typically, adding 1-3ul of EDTA to a 25 µl reaction is sufficient to eliminate non-specific products.


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4. For GC-rich templates (CG percentage is greater than 70%), it's recommended to add 1-5% DMSO to the PCR reaction. Please note that if high DMSO concentration is used, the annealing temperature must be decreased, as DMSO alters the melting point of the primers. 10% DMSO decreases the annealing temperature by 5.5−6.0℃.
5. Eject the sample disc into a PCR reaction instead of an empty tube. Make sure that you see the sample disc in the solution.

## Procedure:

**Prepare PCR master mix → Eject the sample into the PCR reaction → PCR**

## Guidelines for Sampling:

To obtain uniform samples, we recommend using the Harris tools provided in the kit for sampling. The 0.5 mm Harris Uni-Core puncher and the cutting mat can be reused for several hundred times. But these tools should be cleaned between each sample to prevent cross-contamination.

### Sample Type: Plant Leaves

Young leaves are recommended. Fresh plant material is usually the best choice, even though plant material stored at +4℃, frozen or on commercially available cards such as Whatman® 903 and FTA® cards can also be used. Punch out 0.5 mm of sample from the plant leaf using the 0.50 mm Harris Uni-Core puncher supported by the Cutting Mat. Eject the punch disc directly into the PCR reaction (25 or 50 µl in volume). Make sure that you see the sample disc in the solution.

### Sample Type: Plant Seeds

**For very small seeds (such as Arabidopsis):**

Use 1−2 whole seeds and place them directly into the PCR reaction. Make sure that you see the sample in the solution.

**For dehulled seeds:**

Using a clean scalpel, remove the seed coat and cut a small sample of the seed (approximately the size of this dot •) supported by the Cutting Mat. Place the sample directly into the PCR reaction (25 or 50 µl in volume). Make sure that you see the sample in the solution.

### Sample Type: Plant Sample from Commercially Storage Cards, ex: FTA® Cards.

Punch out 0.5 mm of plant sample from the storage card using the 0.50 mm Harris Uni-Core puncher supported by the Cutting Mat. Eject the punch disc directly into the PCR reaction (25 or 50 µl in volume). Make sure that you see the sample disc in the solution.


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**1. Prepare PCR master mix first and then add in the sample.**

Carefully mix and spin down all tubes before opening to ensure homogeneity and improve recovery. The PCR setup can be performed at room temperature. (This protocol serves only as a guideline for PCR amplification. Optional reaction conditions may vary and must be individual determined. Please note that the optimal conditions will depend on the primers, size of the plant sample, the kind of plant used and/or the type of card used. Also some chemicals in plant tissues may alter the $Mg2^{+}$ concentration.)

**Prepare the sample according to different sample type listed in page 3 and eject the sample into the PCR reaction.**

| Component | 25ul / prep | 50ul / prep |
|---|---|---|
| 2X AmpEasy™ Plant PCR Buffer* | 12.5 µl | 25 µl |
| Plant Control Primer Mix (5 µM each) | 1 µl | 2 µl |
| AmpEasy™ Plant DNA Polymerase | 1 µl | 2 µl |
| Plant Seeds or Plant Stored on FTA® Cards / Filter Papers # | 0.5-1 mm² | 0.5-1 mm² |
| $ddH_2O$ | Add to 25 µl | Add to 50 µl |
| **Optional Components for Reaction Optimization** | | |
| $MgSO_4$ Solution§ | 1-3 µl | 2-6 µl |
| EDTA Solution§ | 1-3 µl | 2-6 µl |
| DMSO☆ | 1-5% | 1-5% |

* Includes $Mg^{2+}$ and dNTPs.

# Recommended sample amount is 0.5-1 mm² punch.

§ For high PCR inhibitors in plants, optimization of $Mg^{2+}$ concentration may be required.

☆For GC-rich templates, it's recommended to add 1-5% DMSO to the PCR reaction.

**2. Suggested PCR reaction parameters are as below.**

| Segment | Number of Cycles | Temperature | Time | Cycle Step |
|---|---|---|---|---|
| 1 | 1 | 95℃ | 10 minutes | Cell Lysis. |
| 2 | 40~45 | 95℃ | 30 seconds | Denaturation |
| | | 50~68℃# | 30 seconds | Annealing |
| | | 72 ℃* | 30 seconds | Extension |
| 3 | 1 | 72 ℃ | 1 minute | Final Extension |
| 4 | 1 | 4 ℃ | ∞ | Hold PCR product |

# Optimal annealing temperature is depending on user's primer sequences.

* For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.

**3. Place the PCR tubes or PCR plates in the thermal cycle and start the PCR program.**



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## Recommended Adjustments of $Mg^{2+}$ Concentration

| PCR Inhibitors in Plant | Type of Plant | 25 µL/prep. | |
|---|---|---|---|
| | | $MgSO_4$ | EDTA |
| High | • Rosa rugosa (rose) | 1 µL | 1-3 µL |
| Medium | • Ocimum basilicum (basil)<br>• Vitis uinifera (grape)<br>• Ananas comosus (pineapple)<br>• Phalaenopsis amabilis (moth orchid)<br>• Magnolia denudata (white champak) | 0-1 µL | 0-1 µL |
| Low | • Pachira aquatica (malabar chestnut)<br>• Epipremnum aureum (devil's ivy)<br>• Solenostemon scutellarioides (coleus)<br>• Boussingaultia gracilis (chinses Knotweed)<br>• Citrus reticulata (orange)<br>• Passiflora edulis (passion fruit)<br>• Ipomoea batatas (sweetpotato)<br>• Carica papaya (papaya)<br>• Lycopersicon esculentum (tomato)<br>• Oryza sativa (rice)<br>• Arabidopsis thaliana | 0 µL | 0 µL |


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## Troubleshooting Guide

| No PCR Product |
|---|
| Make sure the plant sample is fresh or under proper storage conditions. Fresh plant material is usually the best choice, even though plant material stored at +4℃, frozen or on commercially available cards such as Whatman® 903 and FTA® cards can also be used. |
| Excessive amounts of crude plant material in a PCR reaction is the major reason for failure. Make sure the punch out sample is 0.5-1 $mm^2$. |
| Make sure that there are no pipetting errors. |
| Use the control primers to perform the control reactions. |
| Check primer design. |
| Optimize annealing temperature according to the primer design. |
| For high PCR inhibitors in plants, optimization of $Mg^{2+}$ concentration may be required. $MgSO_4$ Solution and EDTA Solution are also provided for optimization of $Mg^{2+}$ concentration. For a 25ul reaction, adding 1ul of $MgSO_4$ Solution is equivalent to 1mM $MgSO_4$ in final concentration. We recommend adding 1-3ul for modification. Please note that excess $Mg^{2+}$ may result in spurious PCR products. Also if unspecific products are created, the effective $Mg^{2+}$ concentration can be decreased by adding the chelating agent EDTA. Typically, adding 1-3ul of EDTA to a 25 µl reaction is sufficient to eliminate non-specific products. |
| For GC-rich templates (CG percentage is greater than 70%), it's recommended to add 1-5% DMSO to the PCR reaction. Please note that if high DMSO concentration is used, the annealing temperature must be decreased, as DMSO alters the melting point of the primers. 10% DMSO decreases the annealing temperature by 5.5−6.0℃. |
| **Non-Specific Products with Discrete Bands** |
| Increase annealing temperature or perform a temperature gradient PCR to find the optimal temperature. |
| Shorten extension time. |
| Reduce the total number of cycles. |
| Decrease primer concentration. |
| Design new primers. |


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