ImaSpin® Genomic DNA Extraction Kit –Plant

For purification of genomic DNA from Plant tissue and cells

Precautions

I. Handling Requirements
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

II. Equipment and Reagents to Be Supplied by User

- Ethanol (96–100%)*
- Isopropanol
- 1.5 ml microcentrifuge tubes
- Pipet tips with aerosol barrier
- Vortexer
- Microcentrifuge (with rotor for 1.5 ml tubes) may be required for some samples

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

III. Waste Handling

- Treat waste with the country, federal, state and local regulations.

IV. Important points before use

- Do not use the product if it has expired.
- Add absolute ethanol (see the bottle label for volume) to IW2 Buffer then mix by shaking for a few seconds and tick the checkbox of the label on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Kit Contents

IGP1 Buffer
IGPX1 Buffer
IGP2 Buffer
IGP3 Buffer
IW1 Buffer
IW2 Buffer (Add Ethanol)
Elution Buffer
RNase A
IGP Column
2 ml Collection Tubes

Storage and Stability:

This kit should be stored at room temperature.

Description

ImaSpin® Genomic DNA Extraction Kit –Plant is designed by patented technology for purification of total DNA from plant tissue and cells. The protocol uses buffer contains chaotropic salt to lyse cells and degrade protein. DNA will bind to special column. After washing off the contaminants, the purified DNA is eluted by low salt elution buffer. Purified DNA is suitable for PCR, Real-time PCR, Southern Blotting, RFLP or other enzymatic reactions.

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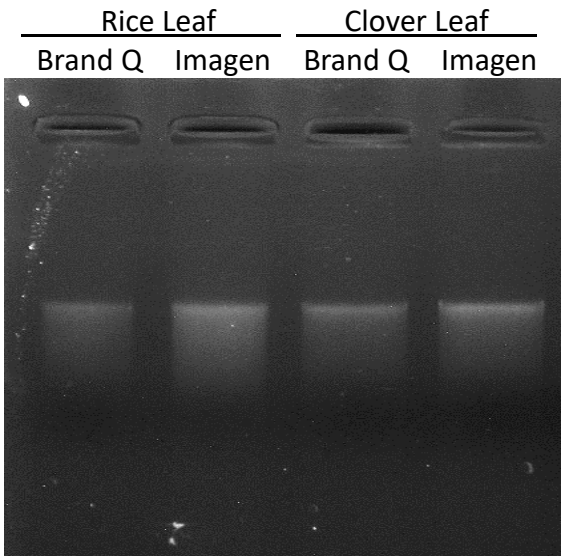

Fig 1. Plant DNA extraction Comparison
Genomic DNA from 50 mg of Rice (*Oryza sativa*) Leaf & Clover (*Oxalis comiculats*) Leaf was extracted using the ImaSpin® Genomic DNA Extraction Kit – Plant & Competitive brand Q. 10 µl from 100 µl eluates of purified genomic DNA was analyzed by electrophoresis on a 1 % agarose gel.

Preparation before using
Add 2 times volume isopropanol to GP3 Buffer immediately prior to initial use.

Protocol Procedure

1. Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue.
2. Grind the sample under liquid nitrogen to a fine powder.
3. Transfer it into a microcentrifuge tube (not provided). Certain plant samples may not require liquid nitrogen treatment.
4. Add 500 µl of IGP 1 (or IGPX 1) Buffer and 5 µl of RNase A (10mg/ml) into the tube and vortaxing to mix well for 10 seconds.
   NOTE: Plant species are extremely diverse in their metabolic components. Large amounts of polysaccharides, carbohydrates, lipids, poly-phenols and proteins may be distributed throughout the plant tissue. These compounds often interfere with DNA binding and extraction. Due to this characteristic of plants, we offer two lysis buffers for optimum performance according to different plant samples.
   IGP1: The standard protocol utilizes IGP1 buffer for plant sample lysis. This buffer system is suitable for most common plant species.
   IGPX1: Suit for dispersing plant samples containing a lot of polyphenols and polysaccharides.
5. Incubate at 65°C for 10 minutes
   NOTE: Inverting the sample occasionally during incubation will facilitate lysis efficiency. Using an auto shaking system is more convenient when incubating samples.
6. At this time, preheat required Elution Buffer (100 µl per sample) in 56 °C (For DNA Elution Step).
7. If insoluble material remains following incubation, centrifuge for 2 minutes at 13,000 rpm (10,000 x g) and transfer all the clarified supernatant to a new microcentrifuge tube (not provided).
8. Add 200 µl of IGP2 Buffer, mix by vortexing and incubate on ice for 3 minutes.

9. If insoluble material remains following incubation, centrifuge for 2 minutes at full speed (10,000 x g, 13,000 rpm) and transfer all the clarified supernatant to a new microcentrifuge tube (not provided).
10. Add a 1.5 volume of GP3 Buffer (make sure isopropanol was added) then vortex immediately for 10 seconds.
    E.g. Add 750 µl of GP3 Buffer to 500 µl of lysate
11. Place an IGP Column in a 2 ml Collection Tube.
12. Apply 700 µl of the mixture (including any precipitation) from previous step to the IGP Column.
13. Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes.
14. Discard the flow-through. Place the IGP Column back in the 2 ml Collection Tube.
15. Apply the remaining mixture to the same IGP Column and centrifuge a 13,000 rpm (10,000 x g) for 3 minutes. Following centrifugation, if the mixture did not flow through the IGP Column membrane, increase the centrifuge time until it passes completely.
16. Add 600 µl of IW1 Buffer to the IGP Column.
17. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IGP Column back in the 2 ml Collection Tube.
18. Add 600 µl of IW2 Buffer (make sure absolute ethanol was added) to the IGP Column.
19. Centrifuge at 13,000 rpm (10,000 x g) for 1 minute, then discard the flow-through. Place the IGP Column back in the 2 ml Collection Tube.
20. Centrifuge for 3 minutes at 13,000 rpm (10,000 x g) to dry the column matrix.
21. Transfer the dried IGP Column to a clean 1.5 ml microcentrifuge tube.
22. Add 100 µl of pre-heated Elution Buffer, TE Buffer or water into the CENTER of the column matrix. NOTE: Standard elution volume is 100 µl. If less sample to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield required, repeat the DNA Elution Step to increase DNA recovery.
23. Let stand for at least 2 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed.
24. Centrifuge at 13,000 rpm (10,000 x g) for 2 minutes to elute the purified DNA.

Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	➢ Ensure absolute ethanol was added to IW2 Buffer and close the bottle tightly after each use to avoid ethanol evaporation. ➢ Reduce the sample material. ➢ Following ethanol addition to the lysate, break up any precipitate as much as possible prior to loading to IGP Column. ➢ Ensure Elution Buffer or water is added into the CENTER of the column matrix. ➢ Elute twice to increase yield.
Eluted DNA does not perform well in downstream applications	➢ Following the Wash Step, dry the IGP column by incubate at 60 °C for 5 minutes. ➢ Use fresh blood, long term storage ample may result in fragmentation of genomic DNA. ➢ Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. ➢ If using water for elution, ensure the water pH is between 7.5 and 8.5. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. DNA eluted in water should be stored at -20 °C to avoid degradation