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EasyMag DNA Purification Kit from plant tissue

Catalog Number: D116-1, D116-2

Table 1. Kit Components and Storage

Kit Component	D116-1 (50 preps)	D116-2 (200 preps)	Storage	Stability
Buffer AP1	25 mL	100 mL	RT	The product is stable for one year when stored as directed.
Buffer AP2	10 mL	40 mL	RT	
Buffer AP3*	15 mL	2×30 mL	RT	
Buffer AW2*	15 mL	2×25 mL	RT	
Buffer AE	15 mL	30 mL	RT	
MagBinding Beads	2.5 mL	10 mL	2-8 ^{o}C	

***** Prior to use, add absolute ethanol to **Buffer AP3, Buffer AW2** according to the bottle label.

Product Description

EasyMag DNA Purification Kit from plant tissue is designed for the rapid and reliable isolation of high-quality genomic DNA from plant, fungal and other tissues that are particularly difficult to lyse or very high in polysaccharide content. This kit is designed for manual or fully automated high throughput preparation of genomic, chloroplast, and mitochondrial DNA. The buffer system is specifically designed to minimize co-purification of polysaccharides and polyphenols. There are no organic extractions, and time-consuming precipitation steps. Purified DNA is suitable for PCR, restriction digestion, next-generation sequencing, and hybridization applications.

Features

- ❖ Fast – DNA purification process in less than 60 min.
- ❖ Safe – No organic extraction, no ethanol precipitation.
- ❖ High-quality – DNA is suitable for a variety of downstream applications.

Things to do before starting

- Buffer AP1 and Buffer AP3 may form precipitates upon storage. If necessary, warm to 50°C to redissolve.
- Buffer AP3 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96-100%) as indicated on the bottle to obtain a working solution.
- Preheat a water bath or heating block to 65°C.
- Prepare RNase A solution (10 mg/mL) and β-mercaptoethanol.

Purification Protocol

This protocol is developed to isolate pure total DNA (genomic, mitochondrial and chloroplast) from fresh, frozen or lyophilized plant or fungal tissue samples.

1. Plant or fungal tissue disruption.

- Liquid nitrogen grinding: Grind the plant or fungal tissue to a fine powder under liquid nitrogen using a mortar and pestle. Transfer the tissue powder (≤100 mg wet weight or ≤20 mg lyophilized tissue) into a 2 mL microcentrifuge tube. Do not allow the sample to thaw.
- TissueRuptor II: Place the tissue sample (≤100 mg wet weight or ≤20 mg lyophilized tissue) into a 2 mL microcentrifuge tube. Add liquid nitrogen to the tube, and freeze the sample for 30 seconds. Keep the sample submerged in liquid nitrogen, and disrupt for approximately 30 seconds at full speed. Allow the liquid nitrogen to evaporate.
- TissueLyser: Place the tissue sample (≤100 mg wet weight or ≤20 mg lyophilized tissue) into a 2 mL safe-lock microcentrifuge tube with 1-2 stainless steel beads. Freeze the tube in liquid nitrogen for 30 seconds. Place the tubes into the TissueLyser Adapter Set, and fix into the clamps of the TissueLyser. Immediately grind the sample for 1 min at 30 Hz. Disassemble the adaptor set, remove the tube, and refreeze in liquid nitrogen for 30 seconds. Reverse the position of the tube within the adaptor set. Grind again for 1 min at 30 Hz.

2. Immediately add 400 µL Buffer AP1 (containing 2% β-mercaptoethanol, prepared freshly) and 10 µL RNase A solution (10 mg/mL) to the disrupted plant or fungal tissue and vortex vigorously.

   Note: Prior to use, add 20 µL β-mercaptoethanol to every 1 mL of Buffer AP1. This will improve the anti-oxidation ability to prevent the oxidation of polyphenols therefore resulting in a low yield of DNA. No tissue clumps should be visible. Vortex or pipet further to remove any clumps. Clumps of tissue will not lyse properly and will therefore result in a lower yield of DNA.

3. Incubate the mixture for 10 min at 65°C. Mix 2-3 times during incubation by inverting tube.
4. Add 130 µL Buffer AP2 to the lysate. Mix and incubate for 5 min on ice.

   Note: This step precipitates detergent, proteins and polysaccharides.

5. Centrifuge the lysate at 14,000 x g for 5 min. Carefully transfer 400 µL the cleared lysate to a new 2 mL microcentrifuge tube. Do not disturb or transfer any of the insoluble pellet.
6. Add 1.5 volumes of Buffer AP3 to the cleared lysate, and mix by pipetting.

   Note: A precipitate may form after the addition of Buffer AP3, pipette up and down several times to disperse the sediment. Buffer AP3 must be diluted with absolute ethanol according to the bottle label before use.

7. Add 50 µL MagBinding Beads to the sample, and vortex at maximum speed for 15-20 seconds.
8. Incubate with shaking at RT for 5 min.
9. Transfer the tube to a magnetic rack for an additional 2 min or until beads pellet and supernatant is cleared. With the tube on the magnetic rack remove the supernatant and discard.
10. Add 600 µL of Buffer AW2 to the tube. Resuspend the beads by pipetting up and down or vortexing at 1,500 rpm for 30 seconds. Replace the tube on the magnetic rack for 2 min or until beads pellet. Remove and discard the supernatant.

    Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.

11. Repeat step 10 for a second wash step.
12. Leave the tube on the magnetic rack, open the cap and air dry the beads at RT for 10-15 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.

13. Add 100-200 µL Buffer AE to the tube, resuspend the beads by vortexing. Incubate at 55ºC for 5 min, vortex for 15 seconds every 1-2 min for 5 min.
14. Replace the tube on the magnetic rack for 2 min or until beads pellet. Transfer the cleared supernatant to a clean tube.
15. Store the DNA at -20ºC.

Troubleshooting

Problem	Possible cause and suggestions
Low yield	• Insufficient disruption: Ensure that the starting material is completely disrupted. • Insufficient lysis: Reduce the amount of starting material and/or increase the amounts of Buffer AP1 and Buffer AP2. • Incorrect binding conditions: Make sure that the correct amount of Buffer AP3 is added to adjust the binding conditions correctly. • DNA still bound to beads: Increase the volume of Buffer AE to 200 µL, and incubate the bead suspension at 55°C for 5 min.
Poor performance in downstream applications	• Salt contamination: Remove any residual liquid with a pipettor after each wash step. • Ethanol contamination: Ensure the beads are completely dried before elution.