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EasySC DNA Purification Kit from plant tissue

Catalog Number: D115-1, D115-2

Table 1. Kit Components and Storage

Kit Component	D115-1 (50 preps)	D115-2 (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	
Mini Column	50	200	RT	
2 mL Collection Tube	50	200	RT	

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

Product Description

EasySC DNA Purification Kit from plant tissue is designed for the rapid and reliable isolation of high-quality total cellular DNA from a wide variety of plant and fungal tissue. Up to 100 mg of wet samples or 20 mg dry samples can be processed in less than 60 minutes. This kit uses a proprietary buffer system to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from plant sample. There are no organic extractions, and time-consuming precipitation steps. Purified DNA is suitable for PCR, restriction enzyme digestion, and hybridization techniques.

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. Connect a DNA Mini Column to a 2 mL Collection Tube. Transfer half mixture including any precipitate that may have formed from step 6 into the DNA Mini column. Centrifuge at 8,000 x g for 1 min.
8. Discard the filtrate and reuse the collection tube. Transfer remaining sample from step 6 to the DNA Mini Column. Centrifuge at 8,000 x g for 1 min.
9. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW2 to the DNA Mini Column, then centrifuge at 8,000 x g for 1 min.

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

10. Repeat step 9 for a second wash step.
11. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

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

12. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube. Add 100 µL Buffer AE preheated to 70°C. Close the lid and incubate at RT for 3 min, then centrifuge at 10,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.

13. Discard the column and store the DNA at -20ºC.

Troubleshooting

Problem	Possible cause and suggestions
Column clogged	• Carryover of particulate material: Ensure that no particulate material is transferred when supernatants are transferred to new microcentrifuge tube prior to addition of Buffer AP3. • Insufficient centrifugation: Increase the *g*-force and centrifugation time. • Lysate too viscous: Reduce the amount of starting material and/or increase the amounts of Buffer AP1and Buffer AP2.
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 the membrane: Increase the volume of Buffer AE to 200 µL, and incubate the column at 50°C for 5 min before centrifugation. • Column matrix lost binding capacity during storage: Add 100 µL 3M NaOH to the column prior to loading the sample. Centrifuge at 10,000 x g for 30 seconds. Add 100 µL water to the columns and centrifuge at 10,000 x g for 30 seconds. Discard the filtrate.
Poor performance in downstream applications	• Salt contamination: Repeat Buffer AW2 wash twice. • Ethanol contamination: incubate the column at 56°C for 5 min to dry the membrane completely.