PRODUCT: **DNAzol®ES (Extra Strength)** August 2023
Cat. No: DN 128
Store at room temperature. Keep tightly closed.

PRODUCT DESCRIPTION

DNAzol® ES is an extra strength DNAzol reagent specifically formulated for the isolation of genomic DNA from plants. The patented procedure (U.S. patent no. 5,945,515) is based on the use of a novel guanidine-detergent lysing solution that allows the selective precipitation of DNA from the lysate [1]. The DNAzol ES protocol is fast and permits efficient isolation of genomic DNA from a variety of plant tissues [2, 3].

In the DNAzol ES procedure, plant samples are pulverized in liquid nitrogen and genomic DNA is solubilized in DNAzol ES. After a brief incubation, the samples are extracted with chloroform and centrifuged. The resulting aqueous phase is collected and DNA is precipitated with ethanol. The pellet is resuspended and washed with a DNAzol ES - ethanol wash solution followed by an ethanol wash and DNA solubilization. The entire procedure can be completed in about 90 minutes and the isolated DNA can be used for Southern analysis, dot blot hybridization, molecular cloning, PCR and other molecular biology and biotechnology applications.

STABILITY: DNAzol ES is stable at room temperature for at least two years after the date of purchase.
HANDLING PRECAUTIONS: DNAzol ES contains irritants. Handle with care, avoid contact with skin, use eye protection (shield, safety goggles). In case of contact, wash skin with a copious amount of water, seek medical attention.

PROTOCOL

Reagents required, but not supplied: ethanol, EDTA, 8 mM NaOH and chloroform.

1. EXTRACTION	- 0.5 g pulverized tissue in 1.5 ml DNAzol ES + 1.5 ml chloroform. 12,000 g x 10 min.
2. DNA PRECIPITATION	- 1 volume aqueous supernatant + 0.75 volume 100% ethanol. 5,000 g x 4 min.
3. DNA WASH	- 1.5 ml DNAzol ES - ethanol wash solution. 5,000 g x 4 min.
	- 1.5 ml 75% ethanol. 5,000 g x 4 min.
4. DNA SOLUBILIZATION	- 8 mM NaOH. 12,000 g x 4 min.

The procedure is carried out at room temperature. Centrifugation can be performed at 4 - 25 C.

1. EXTRACTION

Pulverize plant tissue in liquid nitrogen using a mortar and pestle. Replenish the liquid nitrogen in the mortar 2 - 3 times and continue to grind sample until a fine homogeneous powder is obtained (Note 1). Use a wooden or plastic spatula to transfer the sample to a centrifuge tube containing DNAzol ES. Use 1.5 ml DNAzol ES for each 0.5 g of plant tissue. Mix the pulverized sample with DNAzol ES by inversion until the tissue is thawed and suspended. Store the samples for 5 minutes at room temperature with mixing by inversion or mechanical rotation (Notes 2& 3). *Caution: If liquid nitrogen has not completely evaporated before closing the tube, gas pressure will dislodge the cap resulting in loss of sample.* Supplement the samples with chloroform (1.0 ml of chloroform per 1.0 ml of DNAzol ES) and vigorously shake the mixture for 20 seconds. Do not vortex. Store the mixture for 5 minutes and centrifuge at 12,000 g for 10 minutes. The chloroform extraction removes pigments and insoluble plant debris.
See Note 4 for isolating DNA from plants containing high concentrations of polyphenolics or polysaccharides.

2. DNA PRECIPITATION

Following centrifugation, transfer the upper aqueous phase to a clean tube and precipitate the DNA by mixing 1 volume of aqueous phase with 0.75 volume of ethanol (Note 5). After addition of ethanol, mix the samples by inverting the tubes 8 - 10 times and store them at room temperature for 5 minutes. Sediment precipitated DNA at 5,000 g for 4 minutes and discard the resulting supernatant. Do not exceed a centrifugation force of 5,000 g during the precipitation and washing steps (Steps 2 & 3). Excessive centrifugal force will compress the pellet and make it more difficult to completely solubilize the DNA.
Following ethanol precipitation, plant DNA usually forms coalescent cotton-like strands; however, DNA precipitates may not be visible in extracts that contain large quantities of insoluble polysaccharide.

3. DNA WASH

Prepare the DNAzol ES - ethanol wash solution by mixing 1 volume of DNAzol ES with 0.75 volume of 100% ethanol.
A. Resuspend the DNA pellet in 0.15 - 0.3 ml of EDTA (1 - 10 mM, pH 7-8). Add 5 - 10 volumes of DNAzol ES - ethanol wash solution per volume of EDTA. Store samples for 5 minutes at room temperature and centrifuge at 5,000 g for 4 minutes. See Note 6 for effective removal of polysaccharide and RNA contamination.
B. Decant the DNAzol ES - ethanol wash solution and add 1.5 ml of 75% ethanol. Wash the DNA pellet for 5 minutes by intermittent vortexing and sediment the DNA at 5,000 g for 4 minutes. A second ethanol wash should be employed if plant pigments are visible in the ethanol wash solution. The volume of the ethanol wash solution can be decreased by 50% when washing small DNA pellets.

4. DNA SOLUBILIZATION

Remove the ethanol wash by decanting and store the tubes inverted for 1 - 2 minutes. Remove excess ethanol from the DNA pellet with a micropipette. Do not dry the DNA pellet as this decreases DNA solubility. Solubilize the DNA pellet in 8 mM NaOH or TE buffer (10 mM Tris-HCl + 1 mM EDTA, pH 7.5 - 8.0) by slowly passing the pellet through a pipette tip. Genomic DNA is difficult to solubilize and repeated pipetting may be required. The alkaline solution assures more complete solubilization than water. Incomplete solubilization will result in the loss of DNA during the final centrifugation step. Remove insoluble material

from the solubilized DNA sample by centrifugation at 12,000 g for 4 minutes. Typical yields range from 20 - 200 µg of DNA per g of plant leaf material. Add sufficient solvent to the DNA pellet to achieve a concentration of 0.1 - 0.3 µg DNA/µl.

Alkaline solutions are neutralized by CO_2 from the air. Prepare a fresh solution of 8 mM NaOH every 7 - 14 days from a 2 - 4 M NaOH stock solution that is less than 1 year old. After DNA is solubilized in 8 mM NaOH, adjust the DNA solution to a desired pH by the addition of HEPES buffer.

For 1 ml of 8mM NaOH, use the following amounts of 0.1 M or 1 M HEPES (free acid) to obtain the desired pH:

Final pH	0.1 M HEPES (µl)	Final pH	0.1 M HEPES (µl)	Final pH	1 M HEPES (µl)
8.4	86	7.8	117	7.2	23
8.2	93	7.5	159	7.0	32
8.0	101				

QUANTITATION OF DNA AND RESULTS

Mix an aliquot of the solubilized DNA with 1 ml of water or 1 - 3 mM Na_2HPO_4 and measure the A_{260} and A_{280} of the resulting solution. A slightly alkaline Na_2HPO_4 solution is optimal for the spectrophotometric analysis of RNA and DNA. Laboratory water frequently has an acidic pH which decreases the $A_{260/280}$ ratio (4). Calculate the DNA content assuming that one A_{260} unit equals 50 µg of double-stranded DNA/ml (5). The molecular weight of the isolated DNA ranges from 20 to 100 kb with an $A_{260/280}$ ratio ranging 1.6 - 1.9. The molecular weight of the isolated DNA is influenced by the extent of DNA shearing during tissue preparation.

SIMPLIFIED DNA ISOLATION PROTOCOL (Elimination of chloroform extraction)

The standard DNA protocol has been optimized to consistently isolate high quality DNA from a broad range of plants. Some samples may not require chloroform extraction to isolate high quality DNA. Therefore the isolation procedure can be simplified and shortened as outlined below.

Pulverize plant tissue in liquid nitrogen as described in Step 1 and mix 0.5 g the pulverized tissue with 1.5 ml of DNAzol ES until the tissue is thawed and suspended. Continue to mix the plant - DNAzol ES suspension for 5 - 15 minutes by periodic inversion or mechanical rotation. Remove all particulate and insoluble plant material by centrifugation at 12,000 g for 10 minutes. Carefully collect the supernatant while avoiding disruption of the pellet. The supernatant can be filtered through Nylon mesh if it contains floating pieces of plant tissue. Precipitate the DNA by mixing the supernatant with 0.9 ml of 100% ethanol. The volume of ethanol equals 0.6 volume of the DNAzol ES used to solubilize the pulverized sample. Wash and resolubilize the DNA pellet as outlined in Steps 3 and 4 of the standard isolation protocol.

NOTES

1. Soft tissues (shoots, buds) can be homogenized in a glass-Teflon homogenizer without pulverization in liquid nitrogen.
2. DNA recovery may be improved in some samples when the initial DNAzol ES plant suspension is slowly rotated (5 - 20 RPM) over a period of 15 - 30 minutes prior to chloroform extraction.
3. The pulverized plant tissue suspended in DNAzol ES can be stored for at least one week at room temperature, and at least one month or one year at 4 C or -20 C, respectively. DNA pellets can be stored in 95% ethanol for at least one week at room temperature or for one year at 4 C.
4. DNA quality and recovery from plant tissues containing high concentrations of polyphenolics will be improved when DNAzol ES is supplemented with 2-mercaptoethanol (1% v:v) and polyvinylpyrrolidone (1 - 5% w:v). Add these chemicals to DNAzol ES immediately before the addition of the pulverized tissue and process samples according to the standard protocol.
5. Isolation of a small amount (< 10 µg) of DNA should be performed in the presence of a carrier. Add 5 µl Polyacryl Carrier (MRC Cat. No. PC 152) to the supernatant recovered from the chloroform extraction (Step 2). Proceed with the ethanol precipitation in Step 2 and follow the standard protocol.
6. For samples that contain high levels of contaminating polysaccharides, resuspend the DNA pellet in EDTA as described in Step 3a. To completely remove RNA from the isolated DNA add 50 - 100 ug of RNase to the EDTA suspension and incubate samples for 15 minutes at room temperature. (Heating to 50 C may significantly improve DNA recovery in some samples.) Next, remove insoluble contaminants by centrifugation at 12,000 g for 7 minutes. Transfer the supernatant to a clean tube and precipitate DNA by mixing with 5 - 10 volumes of the DNAzol ES - ethanol wash solution. Centrifuge the DNA at 5,000 g for 4 minutes. Next, wash the DNA pellet with ethanol as described in Step 3b.

REFERENCES

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