invitrogen
by Thermo Fisher Scientific

Plant DNAzol® Reagent

WARNING: Harmful in contact with skin and if swallowed. Contact with acids liberates very toxic gas. Avoid contact with skin and eyes.

Cat. No.: 10978-021 **Size: 100 mL**
CAS No.: 593-84-0 **Store at 15 to 30°C.**

Description:
Plant DNAzol is an extra-strength-DNAzol reagent (patent pending) specifically formulated for the isolation of genomic DNA from plants. The Plant DNAzol procedure is based on the use of a novel guanidine-detergent lysing solution which hydrolyzes RNA and allows the selective precipitation of DNA from the lysate. The Plant DNAzol protocol is fast and permits efficient isolation of genomic DNA from a variety of plant tissues.

In the Plant DNAzol procedure, plant samples are pulverized in liquid nitrogen or homogenized, and genomic DNA is extracted from the homogenate with Plant DNAzol. Following extraction, plant debris is removed by centrifugation and DNA is precipitated from the supernatant with ethanol. The resulting DNA pellet is washed with ethanol and solubilized. The entire procedure can be completed in ~60 min and the isolated DNA can be used for Southern analysis, dot blot hybridization, molecular cloning, PCR, molecular mapping, and other biology and biotechnology applications.

Stability:
Plant DNAzol is stable at room temperature for at least one year after the date of purchase.

Handling Precautions:
Plant DNAzol 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, TE buffer (pH 8.0) or 8 mM NaOH and chloroform.

1.	Extraction	0.3 mL Plant DNAzol + 0.1 g pulverized plant tissue: 0.3 mL chloroform	12,000 × *g*, 10 min
2.	DNA Precipitation	supernatant + 0.225 mL 100% ethanol	5,000 × *g*, 4 min
3.	DNA Wash	0.3 mL Plant DNAzol-ethanol solution 0.3 mL 75% ethanol	5,000 × *g*, 4 min 5,000 × *g*, 4 min
4.	DNA Solubilization	TE buffer (pH 8) or 8 mM NaOH	12,000 × *g*, 4 min

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

1. *Extraction:*
   1.1 Pulverize plant tissue in liquid nitrogen using a mortar and pestle. Replenish the liquid nitrogen in the mortar 2 to 3 times and continue to grind sample until a fine, homogenous powder is obtained. Using a spatula, transfer the frozen powder to a microcentrifuge tube containing Plant DNAzol. (Use 0.3 mL Plant DNAzol for 0.1 g of plant tissue.) Mix the solution thoroughly by gentle inversion a few times and incubate at 25°C with shaking for 5 min. Add 0.3 mL chloroform, mix vigorously, and further incubate at 25°C with shaking for another 5 min. Centrifuge as described below (1,2).
   1.2 Following extraction, centrifuge the extracts at 12,000 × *g* for 10 min and transfer the resulting supernatant, or the aqueous phase after the chloroform extraction, to a fresh tube.
   – For procedures such as PCR which require limited amounts of DNA, addition of chloroform is optional.
   – Protocol is written for isolation of DNA from 0.1 g of plant tissue. For larger amounts of plant tissue, scale up volume of reagents proportionately.
2. *DNA Precipitation*:
   2.1 Following centrifugation, precipitate DNA by mixing the aqueous phase with 0.225 mL of 100% ethanol.
   2.2 After addition of ethanol (2.1), mix samples by inverting the tubes 6 to 8 times and store them at room temperature for 5 min. Sediment precipitated DNA at 5,000 × *g* for 4 min, and remove the resulting supernatant. In some samples, DNA precipitate is not visible before centrifugation.
3. *DNA Wash:*
   3.1 *Plant DNAzol-ethanol wash.* Prepare Plant DNAzol-ethanol wash mixture by mixing 1 volume of Plant DNAzol with 0.75 volume of 100% ethanol. Mix 0.3 mL of Plant DNAzol -ethanol wash solution with the DNA precipitate by vortexing. Store samples for 5 min and centrifuge at 5,000 × *g* for 4 min.
   – When processing large samples (>0.5 g) disperse the DNA pellet in the wash solution with a transfer pipette.
   – The volume of wash solution equals the volume of DNAzol used for the original extraction. When processing plant material with a small amount of contaminants, the volume of the wash solution can be decreased by 50%.
   3.2 *Ethanol wash.* Remove the DNAzol wash solution, and wash the DNA pellet by vigorous mixing with 0.3 mL of 75% ethanol followed by centrifugation at 5,000 × *g* for 4 min.
   – For large samples (>0.5 g), an additional ethanol wash might be necessary to remove chlorophyll and other pigments from the DNA pellet.
4. *DNA Solubilization*:
   Remove the ethanol wash by decanting, store tubes vertically for 1-2 min and remove the remaining ethanol with a micropipette. Air dry the DNA pellet. Dissolve the DNA pellet in 70 µL TE buffer (pH 8.0). If the DNA pellet is difficult to dissolve, use 8 mM NaOH instead of TE buffer. In a typical DNA preparation, the DNA solution is cloudy and may contain insoluble material. This insoluble material is removed by centrifugation at 12,000 × *g* for 4 min.
   – Typical yield is 50 - 300 µg of DNA/g of plant leaf material. Add an adequate amount of TE buffer (pH 8.0) or 8 mM NaOH to achieve a DNA concentration of 0.1 - 0.3 µg/µL.
   – Genomic DNA is difficult to solubilize and repeated pipetting is required for its complete solubilization. Incomplete solubilization will result in loss of DNA during the final centrifugation step.
   – Alkaline solutions are neutralized by CO_2 from the air. Once a month, prepare 8 mM NaOH from a 2 - 4 M NaOH stock solution that is less than 6 months old.
   – If DNA solubilized in 8 mM NaOH, adjust the DNA solution to a desired pH by the addition of HEPES. Use the following amounts of 0.1 M or 1 M HEPES (free acid) per mL of 8 mM NaOH:

Final pH	0.1 M HEPES (µL)	Final pH	1.0 M HEPES (µL)
8.4	86	7.2	23
8.2	93	7.0	32
8.0	101		
7.8	117		
7.5	159		

Quantitation of DNA And Results:
– Mix an aliquot of the solubilized DNA with 1 mL of TE buffer (pH 8.0) or 8 mM NaOH and measure A_{260} and A_{280} of the resulting solution. Calculate the DNA content assuming that one A_{260} unit equals 50 µg of double-stranded DNA/mL (2).
– Molecular weight of the isolated DNA ranges from 20 to 100 kb with the A_{260}/A_{280} ratio ranging 1.6 - 1.9. The molecular weight of the isolated DNA is influenced by the extent of DNA shearing during tissue grinding.

Notes:
1. The isolated DNA may contain degraded RNA. To avoid RNA contamination, add RNase to Plant DNAzol at the beginning of the isolation procedure (100 µg RNase A/mL Plant DNAzol).
2. The isolation procedure can be interrupted and samples can be stored as follows:
   a. Plant DNAzol extract, before or after the initial centrifugation step (step 1.2), 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.
   b. The DNA pellet can be stored in 95% ethanol for at least one week at room temperature or for one year at 4°C.

References:

1. Wilfinger, W.W., Mackey, K., and Chomczynski, P.(1997) *BioTechniques 22,* 474 and 478.
2. Ausubel F.M., Brent R., Kingston R.E., Moore, D.D., Seidmann, J.G., and Struhl, K. (1990) in *Current Protocols in Molecular Biology*, vol 3, p. A.3D.1, John Wiley & Sons, Inc. New York, NY.

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