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Product Information

DNAzol Reagent

Catalog Number	Packaging Size
FP321	100 mL

Storage upon receipt:
- RT
- Protect from light

Product Description

DNAzol Reagent is a complete and ready-to-use reagent for the isolation of genomic DNA from solid and liquid samples of animal, plant, yeast, and bacterial origin. The DNAzol Reagent is a novel guanidine-detergent lysing solution which permits selective precipitation of DNA from a cell lysate. DNAzol Reagent provides a simple, reliable, and efficient DNA isolation method from a variety of samples. The DNAzol Reagent protocol is fast and permits isolation of genomic DNA from a large number of samples of small or large volumes.

During the isolation, a biological sample is lysed (or homogenized) in DNAzol Reagent and the genomic DNA is precipitated from the lysate with ethanol. Following an ethanol wash, DNA is solubilized in water or 8 mM NaOH. The procedure can be completed in 10-30 min with DNA recovery of 70-100%. The isolated DNA can be used without additional purification for applications such as Southern analysis, dot blot hybridization, molecular cloning, and polymerase chain reaction (PCR).

Required materials not supplied

- Ethanol
- 8 mM NaOH

PROTOCOL

1. **Lysis/Homogenization**
   1 ml DNAzol Reagent + 25-50 mg tissue, 1-3 ×10^7 cells, 0.1 ml liquid sample.
2. **Centrifugation (optional)**
   10,000 × *g*, 10 min.
3. **DNA Precipitation**
   Lysate + 0.5 ml 100% ethanol.
4. **DNA Wash**
   1 ml 75% ethanol (2X).
5. **DNA Solubilization**
   8 mM NaOH or water.

This procedure is carried out at room temperature, unless stated otherwise.

1. Lysis/Homogenization

- **Tissues:** Add 1 mL of RNAzol RT Reagent per 25-50 mg of tissue to the sample and homogenize using a hand held homogenizer. Typically, 5-10 strokes are required for complete homogenization. Small amounts (5-10 mg) of soft tissues, such as spleen or brain can be dispersed and lysed by repetitive pipetting with a micropipette. Plant tissues may be efficiently powdered by first freezing in liquid nitrogen before extraction with DNAzol Reagent. Store the homogenate for 5-10 minutes at room temperature.
- **Cells grown in monolayer:** Remove growth media; Add 0.75-1 mL of DNAzol Reagent per 3.5 cm culture dish (10 cm^2) to lyse the cells; Pipet the lysate up and down several times to homogenize.
- **Cells grown in suspension:** Add 1 mL of DNAzol Reagent to 1-3 × 10^7 cells, either in pellet or in suspension (volume < 0.1 mL); Pipet the lysate up and down several times to homogenize. For whole blood up to 100 µL, add 1 mL of DNAzol Reagent to the blood and pipet up and down gently to lyse the cells.
- **Liquid Samples:** Homogenize/lyse liquid samples using 1 mL of DNAzol Reagent per 0.1 mL of a liquid sample; Pipet up and down gently to lyse the sample.
- **Cell Nuclei:** Add 1 ml of DNAzol Reagent to 1-3 × 10^7 cell nuclei, either in pellet or in suspension (volume < 0.1 mL). Lyse the nuclei by inverting or by gently pipetting the mixture.

  To minimize shearing the DNA molecules, mix DNA solutions by inversion; avoid vigorous shaking or vortexing.

2. Centrifugation (optional)

Sediment the homogenate for 10 min at 10,000 ×g at 4-25°C. Following centrifugation, transfer the resulting viscous supernatant to a fresh tube.

This step removes insoluble tissue fragments, RNA and excess polysaccharides from the lysate/homogenate. It is required only for the isolation of DNA from tissues such as liver, muscles and most plant tissues containing a large amount of cellular and/or extracellular material and is also recommended for the isolation of RNA-free DNA.

3. DNA Precipitation

Precipitate DNA from the lysate/homogenate by the addition of 0.5 mL of 100% ethanol per 1 mL of DNAzol Reagent used for the isolation. Mix samples by inverting tubes 5 - 8 times and store at room temperature for 1-3 min. Make sure that DNAzol Reagent and ethanol mix well to form a homogenous solution. DNA should quickly become visible as a cloudy precipitate. Sediment the precipitated DNA by centrifugation at 5,000 ×g for 5 min at 4 - 25°C, carefully remove the supernatant.

4. DNA Wash

Wash the DNA precipitate twice with 1.0 mL of 75% ethanol. At each wash, suspend the DNA in ethanol by inverting the tubes 3 - 6 times. Store the tubes vertically for 0.5 - 1 min to allow the DNA to settle to the bottom of the tubes and remove ethanol by pipetting or decanting.

5. DNA Solubilization

Remove any remaining alcohol from the bottom of a tube using a pipette. Next, dissolve DNA (without drying) in 8 mM NaOH by slowly passing the pellet through a pipette. Alternatively, dissolve DNA in water. However, the alkaline solubilization of DNA occurs faster and assures full solubilization of the DNA precipitate. Add an adequate amount of 8 mM NaOH or water to approach a DNA concentration of 0.2 - 0.3 µg/µl. Typically, add 0.2 - 0.3 mL of 8 mM NaOH or water to the DNA isolated from 10^7 cells or 10-20 mg animal tissue.

The DNA preparations isolated from tissues such as liver, muscles and plants contain some insoluble material (mostly polysaccharides). Remove the insoluble material by centrifugation at 12,000 g for 10 min. Weak 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.

After DNA is 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 1 mL of 8 mM NaOH:

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

6. Quantitation of DNA and Results

Mix an aliquot of the solubilized DNA with 1 mL of 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. The A_{260}/A_{280} ratio of the isolated DNA is within the 1.6 - 1.9 range and with a molecular weight ranging from 20 to 100 kb. The molecular weight of the isolated DNA depends upon the shearing by mechanical forces applied during lysis/homogenization or during solubilization of the DNA precipitate.

For calculation of cell number in analyzed samples or an expected yield of DNA, assume that the amount of DNA per 10^6 of diploid cells of human, rat and mouse origin equals 7.1 µg, 6.5 µg and 5.8 µg, respectively.

Typical yield for animal tissues (µg DNA/mg tissue): liver, kidney or lungs, 3 - 5 µg; skeletal muscle, heart or brain, 1 - 3 µg; plant tissue, 0.3 - 0.8 µg.

The isolated DNA contains partially degraded RNA. If a reduction of the RNA content to less than 3% is necessary, perform the centrifugation step as described in step 2 of the protocol. In Southern analysis, RNA can be digested by supplementing the restriction mix with RNase (1 µg/ml).

NOTES

1. The isolation procedure can be interrupted and samples can be stored as follows: The lysate/homogenate can be stored for 1 month at room temperature or for 10 months at 4 or -20°C. During washes, DNA can be stored in 95% ethanol for at least one week at room temperature or for 3 months at 4°C.

2. For DNA isolation from large blood volumes, first isolate the nuclear fraction and then use DNAzol to extract DNA.

3. A proteinase K digestion can simplify and improve biosafety of the DNA isolation by eliminating aerosol forming devices (homogenizers, blenders). Digest tissue samples (25 -100 mg) for 4 - 24 h at room temperature in 0.5 mL DNAzol supplemented with 10 µL proteinase K (20 mg/mL stock solution). Proteinase K activity in DNAzol is higher at room temperature than at 55°C. Alternatively, perform the digestion in a buffer containing: 50 mM Tris-HCl pH 7.5 - 9.0, 1 mM EDTA, 0.5% SDS and 10 µL proteinase K (20 mg/mL stock solution). Digest 10-150 mg tissue in 0.5 mL of the buffer at 56°C overnight. At the end of the digestion, liquify the tissue completely by gentle pipetting with a disposable transfer pipet and mix 0.1 mL of the digest with 1 mL of DNAzol. After completion of the digestion, proceed according to protocol.