



## 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  $\times 10^7$  cells, 0.1 ml liquid sample.
- 2. Centrifugation (optional)**  
10,000  $\times g$ , 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  $\text{cm}^2$ ) 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  $\times 10^7$  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  $\mu\text{L}$ , 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  $\times 10^7$  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  $\times g$  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  $\times g$  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<sub>2</sub> 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<sub>260</sub> and A<sub>280</sub> of the resulting solution. Calculate the DNA content assuming that one A<sub>260</sub> unit equals 50 µg of double-stranded DNA/mL. The A<sub>260</sub>/A<sub>280</sub> 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<sup>6</sup> 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 ml/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.