

PRODUCT: DNAzol® GENOMIC DNA ISOLATION REAGENT

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Cat. No: DN 127

Store at room temperature. Keep tightly closed.

PRODUCT DESCRIPTION

DNAzol® is a complete and ready to use reagent for the isolation of genomic DNA from solid and liquid samples of animal and plant origin. The DNAzol procedure is based on the use of a novel guanidine-detergent lysing solution that hydrolyzes RNA and allows the selective precipitation of DNA from a cell lysate. Developed by P. Chomczynski (1), DNAzol is a patented DNA isolation method (U.S. patent no. 5, 945, 515) that combines both reliability and efficiency with simplicity of the isolation protocol. The DNAzol protocol is fast and permits isolation of genomic DNA from a large number of samples of small or large volumes (5).

During the isolation, a biological sample is lysed or homogenized in DNAzol 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 minutes with a genomic DNA recovery of 70 - 100%. The isolated DNA can be used, without additional purification, for Southern analysis, dot blot hybridization, molecular cloning, PCR and other molecular biology and biotechnology applications.

STABILITY: DNAzol is stable at room temperature for at least two years after the date of purchase.

HANDLING PRECAUTIONS: 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

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|-------------------------------------|---|
| 1. LYSIS \ HOMOGENIZATION | - 1 ml DNAzol + 25 - 50 mg tissue, 10 ⁷ cells or 0.1 ml liquid sample. |
| 2. CENTRIFUGATION (optional) | - 10,000 g x 10 minutes. |
| 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. |

The procedure is carried out at room temperature, unless stated otherwise. Reagents required: ethanol and 8 mM NaOH.

1. LYSIS / HOMOGENIZATION

A. **TISSUES.** Homogenize tissues in a hand held glass-Teflon homogenizer. Use a loosely fitting homogenizer, with a tolerance greater than 0.1 - 0.15 mm. Homogenize 25 - 50 mg tissue in 1 ml of DNAzol by applying as few strokes as possible. 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. Store the homogenate for 5 - 10 minutes at room temperature.

B. **CELLS.** Cells grown in monolayer should be lysed directly in a culture dish. Pour off media, add DNAzol and pass the cell lysate several times through a pipette. Add 0.75 - 1.0 ml of DNAzol per 10 cm² culture plate area.

Cell pellets or suspensions, add 1 ml of DNAzol to 10⁷ cells (volume < 0.1 ml) and lyse the cells by repeated pipetting.

Cell nuclei, add 1 ml of DNAzol to 1 - 3 x 10⁷ cell nuclei (volume < 0.1 ml) and lyse the nuclei by inversion or repeated pipetting.

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

Please see Note 5 for a description of an optional proteinase K digestion procedure.

2. CENTRIFUGATION (Optional)

Sediment the homogenate for 10 minutes 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, partially hydrolyzed 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 used for the isolation. Mix samples by inverting tubes 5 - 8 times and store at room temperature for 1 - 3 minutes. Make sure that DNAzol and ethanol mix well to form a homogenous solution. DNA should quickly become visible as a cloudy precipitate. Remove the DNA precipitate by spooling with a pipette tip. Swirl the DNA onto the tip and attach it to the tube wall near the top of the tube by gently sliding the DNA off the tip. Alternatively, transfer the DNA to a clean tube. Store the tubes upright for about 1 minute and remove from the bottom of the tubes the remaining lysate/homogenate.

Degraded DNA and small quantities of DNA (< 15 µg) do not spool onto a pipette tip. In this case, sediment the precipitated DNA by centrifugation at 5,000 g for 5 minutes at 4 - 25 C.

4. DNA WASH

Wash the DNA precipitate twice with 0.8 - 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 minutes to allow the DNA to settle to the bottom of the tubes and remove ethanol by pipetting or decanting.

If necessary, sediment the DNA pellet at 1,000 g for 1 - 2 min at 4 - 25 C. To further remove contaminants when isolating DNA from tissues, the first ethanol wash can be replaced with wash in a solution containing 70% DNAzol and 30% ethanol.

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 minutes. Weak alkaline solutions are neutralized by CO₂ 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.

Adjustment of pH in DNA samples solubilized in 8mM NaOH.

For 1 ml of 8 mM NaOH use the following amounts of 0.1 or 1M HEPES (free acid)

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 8 mM NaOH, 1 - 3 mM Na₂HPO₄ or water and measure A₂₆₀ and A₂₈₀ of the resulting solution. Calculate the DNA content assuming that one A₂₆₀ unit equals 50 µg of double-stranded DNA/ml. The A₂₆₀/A₂₈₀ 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 10⁶ of diploid cells of human, rat and mouse origin equals 7.1 µg, 6.5 µg and 5.8 µg, respectively (2). 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. For cell nuclei isolation we recommend a simple and efficient citrate method based on homogenization of tissues in 40 mM sodium citrate-1% Triton X-100 solution. The method is described in detail in MRC Technical Bulletin # 2.
2. 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 C 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.
3. Isolation of small amount (< 15 µg) of DNA should be performed in the presence of a carrier. Lyse or homogenize sample in 0.5 ml of DNAzol supplemented with 5 µl of Polyacryl Carrier (Cat. no. PC 152). Follow the protocol by precipitating the DNA-carrier mix with ethanol (# 3, Precipitation).
4. For DNA isolation from large blood volumes, first isolate the nuclear fraction and then use DNAzol to extract DNA. For small volumes of blood (1 - 50 µl), use 250 - 500 µl of DNAzol supplemented with 2 - 3 µl of Polyacryl Carrier (3).
5. 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 CaAcetate, 0.5% SDS and 10µl 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 (4). After completion of the digestion, proceed according to protocol. MRC Technical Bulletin #5 contains additional information on the use of proteinase K for isolation of DNA from mouse tails.

REFERENCES

1. Chomczynski P, Mackey K, Drews R and Wilfinger W (1997) DNAzol: A reagent for the rapid isolation of genomic DNA. *BioTechniques*, 22, 550-553.
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3. Mackey K, Williams P, Seim S and Chomczynski P (1996) The use of DNAzol for the rapid isolation of genomic DNA from whole blood. *Biomedical Products, Supplement* 13-15.
4. Liska V and Ruprecht RM (1999) Isolation of high-molecular weight genomic DNA from intact biohazardous mammalian tissues. *Biotechniques* 26, 62-66.
5. Chomczynski P, Wilfinger W, Mackey K (1998) Isolation of Genomic DNA from Human, Animal, and Plant Samples with DNAzol Reagents.