For ***in vitro*** laboratory use only

Ecoli

DNAzol Genomic DNA Isolation Reagent

a complete and ready to use reagent for the isolation of genomic DNA

DP3001 50ml
DP3002 100ml

Storage Conditions: 15 to 30°C

WARNING:

Harmful in contact with skin and if swallowed. Contact with acids produces toxic gas. Avoid contact with skin and eyes.

Description:

DNAzol Reagent (Genomic DNA Isolation 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 procedure is based on the use of a novel guanidine-detergent lysing solution which permits selective precipitation of DNA from a cell lysate. Since first proposed by Cox (1), the isolation of genomic DNA with guanidine salts has been the subject of numerous reports and commercial applications. Developed by Chomczynski, DNAzol Reagent is an advanced DNA isolation reagent that combines both reliability and efficiency with simplicity of the isolation protocol. The DNAzol Reagent protocol is fast and permits isolation of genomic DNA from a large number of samples from 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).

Stability:

DNAzol Reagent is stable at 15 to 30°C for at least one year after the date of purchase.

Procedure:

1. **Lysis/Homogenization** 1 mL DNAzol Reagent + 25-50 mg tissue, 1-3 x 10^7 cells, or 0.1 ml liquid sample.
2. **Centrifugation (optional)** 10,000 x 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

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

Unless stated otherwise, the procedure is carried out at room temperature.

1. Lysis of cells and nuclei:

a. Cells grown in monolayer: Add 0.75-1.0 ml of DNAzol Reagent per 10 cm^2 culture plate area. Lyse the cells by agitating the culture plate and gently pipette the lysate into an assay tube.

b. Cell Pellets or Suspensions: Add 1 ml of DNAzol Reagent to 1-3 x 10^7cells, either in pellet or in suspension (volume < 0.1 ml). Lyse the cells by gently pipetting. For whole blood up to 100 microliters, add 1 ml of DNAzol to the blood and pipette up and down gently to lyse the cells. For whole blood (>100 µl), pellet the cells and wash them with 0.9% NaCl. Pellet the cells again and resuspend them in one volume of cold (4°C) hypotonic solution (20 mM Tris HCl, pH 8.0, 10 mM EDTA). Pellet the cells at 4,000 rpm for 10 min (4°C). Discard the supernatant and add 1 ml DNAzol per 1-3 x 10^7cells. Lyse the cells by gently pipetting.

c. Cell Nuclei: Add 1 ml of DNAzol Reagent to 1-3 x 10^7 cell nuclei, either in pellet or in suspension (volume < 0.1 ml). Lyse the nuclei by inverting the assay tube or by gently pipetting the mixture.

d. To minimize shearing of the DNA molecules, pipette DNA solution using wide bore pipette tips. Prepare wide bore pipette tips by cutting 2-3 mm from the ends of plastic pipette tips. Mix DNA solutions by inversion; avoid shaking or vortex for mixing.

Homogenization of tissues:

Homogenize tissue samples in a hand held glass/Teflon® homogenizer. Use a loosely fitting homogenizer, with a tolerance of 0.1-0.15 mm or higher.Homogenize 25-50 mg tissue in 1 ml of DNAzol Reagent 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 into smaller fragments and lysed by repetitive pipetting with a micropipette. Plant tissues may be efficiently powdered by first freezing in liquid nitrogen or dry ice/ethanol before extraction with DNAzol.

2. Centrifugation (optional):

Sediment the homogenate for 10 min at 10,000 rpm at 4°C or room temperature. 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. This process is recommended in order to minimize RNA carry-over into the 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 inversion and store them at room temperature for 1-3 min. 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). Carefully decant the supernatant, leaving the DNA pellet near the top of the tube. Place the tubes upright for 1 min and aspirate the remaining lysate/homogenate from the bottom of the tubes. If extensive pipetting is used to facilitate lysis/homogenization before precipitation with ethanol, the resulting sheared DNA will not spool. The same is true for small quantities of DNA (<15 µg). In this case, centrifugation at 4,000 g for 1-2 min at room temperature or 4°C will pellet the DNA.

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 min to allow the DNA to settle to the bottom of the tubes and remove completely ethanol by pipetting or decanting.

5. DNA Solubilization:

a. Air dry the DNA by storing in an open tube for 30-60 seconds after removing the ethanol.

(***If the DNA is exposed to air for more than mentioned time, it will be much more difficult to dissolve it.) Dissolve the DNA in 8 mM NaOH by slowly passing the pellet through a pipette tip. Use of the 8 mM NaOH assures full solubilization of the DNA precipitate. Add an adequate amount of the 8 mM NaOH to approach a DNA concentration of 0.2-0.3 µg/µl. Typically add 0.2-0.3 ml of 8 mM NaOH to the DNA isolated from 10^7cells or 10-20mg of animal tissue. DNA will not be fully solubilized in TE or water. (The resolubilization of DNAzol-isolated DNA is low in Tris buffers. Therefore the use of 8 mM NaOH is highly recommended.) DNA is stable in 8 mM NaOH for several months at 4°C and greater than one year at –20°C.

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

c. 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 six months old.

d. 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:

a. Mix an aliquot of solubilized DNA with 1 ml of 8 mM NaOH 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 per ml.

b. For calculations of a cell number in analyzed samples or an expected yield of DNA, assume that the amount of DNA per 10^6 diploid cells of human, rat,and mouse origin equals 7.1 µg, 6.5 µg, and 5.8 µg, respectively (2).

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

d. 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 its shearing by mechanical forces applied during lysis/homogenization or during solubilization of the DNA precipitate.

e. 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 A (1 µg/ml).

Notes and Comments:

The isolation procedure can be interrupted and samples can be stored as follows:The lysate/homogenate can be stored for 18 hours at 15 to 30°C or for three daysat 2 to 8°C (refer to Step 1).During washes, DNA can be stored in 95% ethanol for at least one week at 15 to30°C or for three months at 2 to 8°C (refer to Step 4).

References:

1. Cox, R.A. (1968) Methods in Enzymology (Grossmann, L. and Moldave, E., Eds.)Vol. 12, Part B, pages 120-129, Academic Press, Orlando, FL.

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 2, page A.1.5.,John Wiley & Sons, Inc. New York, NY