

**STANDARD OPERATING PROCEDURE:
GENOMIC DNA ISOLATION USING DNazol®¹**

PI: _____	Room & Building: _____
Department: _____	Research Group: _____
Date: _____	Pertains to Lab Protocol: _____

PROCEDURE

If available, attach the experimental protocol for genomic DNA isolation using DNazol®.

MATERIALS & HAZARDS

Principal Materials Used	Corrosive	Irritant	Sensitizer	Reproductive toxin	Acutely Toxic	Carcinogen	Flammable	Combustible	Water-Reactive	Shock-Sensitive	Pyrophoric	Oxidizer	Biotoxin	Other Comments:
DNazol		X						X						
Ethanol		X		X			X							See comment 1, below.
Sodium hydroxide	X	X												

Other comments:

1. May affect central nervous system.

ENGINEERING/VENTILATION CONTROLS

All steps involved in the genomic DNA isolation using DNazol® should be performed in a chemical fume hood.

RECOMMENDED PERSONAL PROTECTIVE EQUIPMENT

The level of skin and eye protection should be selected based on the potential for splash and exposure to occur.

Incidental Contact (minimum potential for splash & exposure):

- ✓ Safety glasses with side shields
- ✓ Double latex gloves
 - Double-gloved is recommended given the diversity of chemical hazards involved and the corrosive characteristic of sodium hydroxide.
 - Immediately replace with new gloves when splash occurs.
 - The chemical breakthrough time for latex gloves once in contact with ethanol is **37 minutes**.
- ✓ Protective clothing (e.g. non-porous lab coat, closed-toed footwear)

RECOMMENDED PERSONAL PROTECTIVE EQUIPMENT *(continued)*

Extended Contact (when using or transferring large quantities, or for spill clean-up):

- ✓ Chemical splash goggles
- ✓ Face shield (if not working in a fume hood or if hood's sash is not in the down position)
- ✓ Double latex gloves
 - Double-gloved is recommended given the diversity of chemical hazards involved and the corrosive characteristic of sodium hydroxide.
 - Immediately replace with new gloves when splash occurs.
 - The chemical breakthrough time for latex gloves once in contact with ethanol is **37 minutes**.
- ✓ Chemical resistant apron/smock/lab coat (PE or PVC)
 - Avoid using the traditional cotton-polyester white lab coat, which readily collects/absorbs compounds.
- ✓ Closed-toed non-porous footwear

ADDITIONAL PRECAUTIONS

- Ensure tubes are capped whenever contents are being mixed by hand or vortex.
- Place a paper towel moistened with appropriate disinfectant over the top of the tube when homogenizing the tissues. This will help to reduce exposure to droplets/aerosols.
- Use capped polypropylene tubes when centrifuging the homogenate. Wait at least 10 minutes before opening the centrifuge to avoid exposure to aerosols.

MATERIAL SAFETY DATA SHEETS

MSDSs are available electronically via EHS Department's Web page:

http://www.uos.harvard.edu/ehs/onl_msd.shtml. An option, but consider collecting in a binder the MSDSs that arrive with each order.

WASTE

Refer to the *Harvard Longwood Laboratory Waste Guide* at

http://www.uos.harvard.edu/ehs/longwood/HarvardLongwoodLabWasteGuide_v1.0.pdf.

EMERGENCY PROCEDURES

Refer to the emergency flip chart titled "*EHS Procedures and Response Guidelines*," posted in each laboratory.

References:

1. Molecular Research Center, Inc., 1995, "TRI REAGENT BD – RNA/DNA/Protein Isolation Reagent for Blood Derivative", Cincinnati, OH.

ATTACHMENT
PROCEDURE FOR GENOMIC DNA ISOLATION USING DNAzol®
<http://www.mrcgene.com/dnazol.htm>

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 an advanced 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, polymerase chain reaction (PCR)* and other molecular biology and biotechnology applications.

* Patents granted to Hoffmann LaRoche Corporation.

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 and seek medical attention.

PROTOCOL

The protocol includes the following steps:

- 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 performed 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 pipeting 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 this optional 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.

<u>Adjustment of pH in DNA samples solubilized in 8 mM NaOH</u>			
For 1 ml of 8 mM NaOH, use the following amounts of 0.1M or 1 M HEPES (free acid)			
<u>Final pH</u>	<u>0.1 M HEPES (µl)</u>	<u>Final pH</u>	<u>1 M HEPES (µl)</u>
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 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 10^6 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 AND COMMENTS

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 [Technical Bulletin # 2](#). Call or fax MRC to receive this bulletin.

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 (Step# 3, DNA 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](#) . These protocols have been described (3) and reprints can be obtained by contacting MRC.
5. The 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 proteinase K (100 µg/ml). Proteinase K activity in DNAzol is higher at room temperature than at 55 C. Alternatively, perform the digestion in a buffer containing: 10mM Tris-HCl, pH 7.6, 20 mM NaCl, 100 mM Na-EDTA (pH 7.5 - 8), 1% sodium laurylsarcosine and 0.1 mg/ml of proteinase K. 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.
2. Ausubel F., Brent R., Kingston R., Moore D., Seidman J. and Struhl K. 1990. *Current Protocols in Molecular Biology*, 2, A.1.5., John Wiley & Sons, Inc. New York, NY.
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 R. 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. *Biotechnology International*, 185-188.