Hangzhou Simgen Biotechnology Co., Ltd.
5th floor, Building No. 4, No. 8 Xiyuan 1st Road, Hangzhou, P. R. China.
Pc: 310030 Tel: +86-571-87381295 Fax: +86-571-87381295

DNAzol Instructions

Composition

Cat. No.	3107050	3107100
DNAzol	50 ml	100 ml
Instructions	1	1

Storage

The product should be stored at 0-30℃ and has an expiration date of 2 years.

Technical Support

R&D Department, Hangzhou Simgen Biotechnology Co., Ltd.
E-mail: technical@simgen.cn, Tel: 400-0099-857.

Introduction

DNAzol is a complete, ready-to-use DNA extraction reagent for extracting genomic DNA from solid or liquid samples of animal, plant, yeast, and bacterial origin. DNAzol is a lysis buffer solution formulated with guanidine salts and a variety of detergents that selectively lyses and releases DNA from cell lysates, and the DNA in the resulting supernatant is then precipitated with ethanol. DNAzol is a state-of-the-art DNA extraction reagent that combines high DNA recovery efficiency with simple procedures.
DNAzol is a quick and easy procedure for extracting genomic DNA from various sample volumes. During extraction, biological samples were lysed in DNAzol, and genomic DNA was precipitated from the lysate with ethanol and washed with 75% ethanol to dissolve the DNA in 8 mM NaOH. The process can be completed in 10-30 min with 70-100% DNA recovery. The extracted DNA can be used for applications such as Southern analysis, dot blotting, molecular cloning, and polymerase chain reaction (PCR) without additional purification.

Equipment And Reagents to Be Supplied by Users

1. 1.5 ml centrifuge tubes/2 ml centrifuge tubes, pipettes and tips
2. Disposable gloves and protective equipment and tissues
3. Microcentrifuge (s) (with rotor for 1.5 ml and 2 ml centrifuge tubes)
4. Absolute ethanol, 75% ethanol, 8 mM NaOH

Preparation Before Use

DNAzol is corrosive, avoid contact with skin and eyes, wear gloves and goggles.

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Protocol

This procedure is designed to extract DNA from 1 ml DNAzol, and if DNA is extracted from large volume samples, the amount of DNAzol and absolute ethanol must be increased proportionally.

1. Treatment of samples from different sources:

Human or animal tissue:

Grind about 100 mg of tissue to powder form in a mortar with liquid nitrogen, weigh 20-50 mg of human or animal tissue in a 1.5 ml centrifuge tube pre-cooled with liquid nitrogen, add 1 ml DNAzol, do not discard the pipette tip, and directly pipette the sample several times with the tip to lyse it, and proceed to step 3.

Cultured animal cells:

Adherent cells: Add 1 ml DNAzol per 10 cm^2 of cultured cells (e.g., a cell culture dish with a diameter of 3.5 cm, add 1 ml DNAzol after discarding the medium), do not discard the pipette, pipette the cells several times to lyse the cells, transfer the homogenate into a 1.5 ml centrifuge tube, and proceed to step 3.

Suspension culture: Collect 1~3×10^7 cells with 1.5 ml centrifuge tubes, add 100 µl PBS solution, vortex and shake until the cells are all suspended, add 1 ml DNAzol, and directly pipette the cells several times with the tip to lyse the cells, and proceed to step 3.

Plant tissues/plant cells/yeasts/bacteria:

Grind about 100 mg of the sample to powder form with liquid nitrogen in a mortar, then weigh 20-50 mg of the powdered tissue with a 1.5 ml centrifuge tube pre-cooled with liquid nitrogen, add 1 ml DNAzol, do not discard the pipette tip, and directly pipette the sample several times with the tip to lyse it, and proceed to step 3.

* Lysozyme treatment can also be used for bacterial samples: centrifuge at 12000 rpm for 30 sec to collect 1 ml bacterial samples, discard the supernatant, add 50 µl pure water, vortex to suspend and disperse bacteria, add 50 µl 50 mg/ml lysozyme solution, invert and mix well, and incubate at 37℃ for 30 min. After the incubation, the bacterial solution was first vortex and shaken for 10 sec to suspend and disperse. Then add 1 ml DNAzol, pipette several times until the solution was colorless and transparent, and then proceeded to step 3.

Liquid samples (e.g. fresh/frozen anticoagulated whole blood, body fluids, etc.):

Add 1 ml DNAzol to every 100 µl liquid sample, do not discard the tip, and pipette the sample several times to lyse it, and proceed to step 3.

* If there is enough fresh anticoagulated whole blood, red blood cell lysate (Simgen Cat. No.: 9000500) to extract the leukocytes, add 100 µl PBS solution, vortex until the cells are all suspended, add 1 ml DNAzol, and directly pipette the cells several times with the tip to lyse the cells, and proceed to step 3.

2. Optional step: Centrifuge at 10,000 ×g for 10 min and transfer the supernatant into a 1.5 ml centrifuge tube.

* It is required if the sample is not transparent after the sample was mixed with DNAzol.
* This step removes insoluble tissue debris, RNA, and polysaccharides from the lysate. It is only used to extract DNA from liver, muscle, and plant tissues that contain large amounts of extracellular material.
* This step is recommended to minimize RNA retention in the DNA.

3. Add 500 µl absolute ethanol, invert several times, centrifuge at 4,000×g for 2 min.

* If the DNA content in the sample is known to be relatively low (less than 2 µg), the speed can be increased to 12,000×g for 15 min to improve the DNA yield.

4. Discard the supernatant, add 1 ml 75% ethanol, close the lid, gently invert the centrifuge tube 4~6 times, and centrifuge at 7500×g for 5 min.

5. Repeat step 4 once.

6. Discard the supernatant, close the lid, and spin down the ethanol on the tube wall to the bottom of the tube. Discard the residual ethanol with a 200 µl tip, retaining the white DNA pellet in the bottom of the tube. There is no need to dry the DNA.

7. Add 50-200 µl 8 mM NaOH, the dissolved DNA in 8 mM NaOH is stable for several months at 4℃ and more than a year at -20℃.

* DNA must be solubilized with a weak base as the precipitated DNA may not be soluble in water or in Tris buffer.
* Avoid vigorously shaking or vortexing, dissolve the DNA by inverting the tube, which can reduce the shearing of genomic DNA.
* To minimize fragmentation of DNA molecules, it is recommended to use wide-bore pipette tips to transfer DNA solutions. Wide-bore pipette tips are made by cutting off the end of the pipette tip 2-3 mm.
* If the DNA (especially from tissues) contains insoluble colloids (membrane fragments, etc.) or the DNA solution is cloudy, centrifuge at 12,000 ×g for 10 min and transfer the DNA-containing supernatant to a new tube.
* If the DNA purity obtained is poor (A260/280 ratio < 1.70), Simgen DNA Purification Kit (Cat. No. 2101050) can be used to purify DNA before use.

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Whole blood samples can be processed to improve DNA yield by following steps:

1. **Add 500 µl whole blood and 1 ml DNAzol reagent to a 2 ml centrifuge tube, close the lid, and mix well by vortexing or shaking.**
2. **Add 400 µl isopropanol, close the lid, mix well by vortexing or shaking, incubate at room temperature for 5 min.**

* The volume of isopropanol is equal to 0.4 times the volume of DNAzol reagent.

* Mix the DNAzol-blood lysate with isopropanol to lyse protein aggregates and improve the quality of isolated DNA.

3. **Centrifuge at 6000×g for 6 min.**
4. **Discard the supernatant, add 500 µl DNAzol reagent, and mix well by vortexing or shaking until the DNA pellet is completely dispersed. Centrifuge at 6000×g for 5 min.**
5. **Discard the supernatant, add 1 ml 75% ethanol to wash the DNA pellet, and centrifuge at 6000×g for 5 min.**

* If there is blood residue on the lid and mouth of the centrifuge tube, carefully wipe it away with a cotton swab.

6. **Discard the supernatant, close the lid, and spin down the ethanol on the tube wall to the bottom of the tube. Discard the residual ethanol with a 200 µl tip, leaving the white DNA pellet on the bottom and wall of the tube. No drying of DNA is required.**
7. **Add 200 µl 8 mM NaOH and incubate at room temperature for 3-5 min. Repeated pipetting or vortexing can help dissolve DNA.**