MB501-100 ml DNA- XPress™ Reagent

100 ml solution containing Guanidinium isothiocyanate

Intended Use

Recommended for isolation of DNA from human (blood samples), tissue, plant, bacteria, yeast and samples of viral origin.

Storage Conditions

DNA-XPress™ Reagent can be stored at room temperature (15-25°C) for upto 1 year without showing any reduction in performance. However, storing at lower temperatures will cause precipitation of guanidinium isothiocyanate from the solution. If the reagent is warmed (55-65°C), it should resolubilize instantly.

Materials needed but not provided

- Ethanol (96-100%)
- 95% Ethanol
- 8mM NaOH (freshly prepared)
- Chloroform (for plants only)
- 10X RBC Lysis Solution (for whole blood only) (Product code: R075)
- RNase A Solution-20mg/ml (Optional) (Product code: DS0003)
- HEPES (Product code: MB016)
- Proteinase K Solution-20mg/ml (Product code: DS0013)
- Digestion buffer (for mouse tail): 50 mM Tris pH 8.0, 100 mM EDTA, 0.5% SDS (Product code: ML007- 20% SDS Stock solution)
- Molecular Biology Grade Water (Product code: ML024)
- Nuclease free microcentrifuge tubes
- Heating block or water bath (for bacterial cells, yeast, plant and mouse tail)

Introduction

DNA-XPress™ Reagent is a non-organic ready to use reagent for the isolation of genomic DNA from blood, tissue, plant, bacteria, yeast and samples of viral origin. The protocol is rapid and permits isolation of genomic DNA from large number of samples of small or large volumes. The DNA obtained can be further used for downstream applications such as PCR, molecular cloning, restriction enzyme digestion, southern blotting, dot blot hybridization and other molecular biology applications.

Principle

DNA-XPress™ Reagent is designed for rapid purification of DNA from different samples. The DNA-XPress™ procedure is based on the use of a novel guanidine-detergent lysing solution that hydrolyzes RNA and promotes the selective precipitation of DNA from the cell lysate. This advanced DNA isolation procedure is based on Chomczynski method, which combines reliability and efficiency with the simplicity of a fast isolation protocol.

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Concentration, yield & purity of DNA

The final preparation of genomic DNA isolated with DNA-XPress™ Reagent is upto 20-50 kb. Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use 8mM NaOH or Molecular Biology Grade Water (ML024) to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm, and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall between 0.1 and 1.0, the 320 nm absorbance is used to correct for background absorbance. An absorbance of 1.0 at 260 nm corresponds to approximately 50μg/ml of DNA. The $A_{260}–A_{320}$ /$A_{280}–A_{320}$ ratio should be 1.6-1.9. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. DNA purified by DNA- XPress™ Reagent is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

General Preparation Instructions

1. Preheat a water bath or heating block to 60°C (For bacteria, yeast and plant samples).
2. Dilute the 10X RBC Lysis Solution to 1X concentration before use (For whole blood only).

Centrifugation

All centrifugation steps are carried out in conventional laboratory centrifuge e.g. Beckman CS-6KR, Heraeus Varifuge 3.0R, or Sigma 6k10 with fixed angle rotor. All centrifugation steps are performed at room temperature and are given in *g*, the correct rpm can be calculated using the formula:

$$RPM = vRCF/1.118 \times 10^{-5}\, r$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g), r = radius of the rotor in cm and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force

Specimen Handling and Collection

For Whole blood

Collect whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (if to be used for future). Ensure that the blood sample is at room temperature before beginning the protocol.

For tissues

Collect human/animal cells, tissues, blood sample in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage. Ensure that the tissue is at room temperature before beginning the protocol.

Types of Specimen

Clinical samples: blood samples, stool, sputum, urine, wound exudates and viral cultures

Procedure

Samples may be either fresh or frozen (except for blood). DNA obtained may contain some amount of degraded RNA; the concentration of RNA is less than 3% of the DNA. If RNA-free DNA sample is required, apply RNase A Solution (DS0003) to the DNA sample.

The procedure can be halted at the following stages during isolation:

1. The lysate containing DNA-XPress™ Reagent can be stored at room temperature (15-25°C) for 18 hours or at 4°C or -20°C for 9 months.

2. During the washing steps, DNA can be stored in 95% ethanol for atleast 1 week at room temperature (15-25°C), or 3 months at 4°C.
3. For long-term storage of high molecular weight DNA, re-precipitate the DNA and store in ethanol at 4°C.

Protocol for Genomic DNA Isolation

A. Homogenization

The samples should be gently, but thoroughly, homogenized with DNA-XPress™ Reagent. Homogenization can be achieved by repetitive pipetting with a Pasteur pipette. The sample will become viscous due to the release of high molecular weight genomic DNA. Pipetting too vigorously may shear the genomic DNA. All samples should be held at room temperature (15-25°C) for 5 minutes, unless stated otherwise.

1. **Tissue**

Gently homogenize the tissue sample in the reagent. Use 1ml of DNA-XPress™ Reagent for 50 mg of tissue.

2. **Cells**

a. Cells grown in a monolayer: These should be lysed directly in the culture dish by addition of 1ml of DNA-XPress™ Reagent per 10cm^2 area of culture dish. Discard the media, add DNA-XPress™ Reagent and pass the cell lysate several times through a pipette.
b. Cells grown in suspension: 1ml of DNA-XPress™ Reagent per 10^7 cells should be used. The cells should be pelleted and then lysed.
c. Cell nuclei: 1ml of DNA-XPress™ Reagent per 10^7 cell nuclei should be used. Mix the sample by inverting the tube or repeated pipetting.

3. **Bacterial cells**

a. Gram-positive bacteria: 1ml of DNA-XPress™ Reagent per 10^7 cells should be used. Freeze the cells in liquid nitrogen and grind to a fine powder using a mortar and pestle or homogenizer and incubate for 1 hour at 60°C.
b. Gram-negative bacteria: 1ml of DNA-XPress™ Reagent per 10^7 cells should be used to sediment the cells. Lyse the cells by repetitive pipetting and incubate for 15 to 60 minutes at 60°C.

4. **Yeast**

1ml of DNA-XPress™ Reagent per 10^7 cells should be used to sediment the cells. Homogenize and incubate for 15 to 60 minutes at 60°C.

5. **Plant**

1ml of DNA- XPress™ Reagent per 50 to 200 mg of plant should be used. Freeze the plant sample in liquid nitrogen and grind it to a fine powder using a mortar and pestle or homogenizer and incubate for 1 hour at 60°C. Proceed with section B of the protocol.

6. **Whole Blood**

DNA can be isolated from fresh whole blood (treated with citrate, heparin or EDTA). To 1ml of whole blood, add 3ml of 1X RBC Lysis Solution (R075). Mix gently at room temperature (15-25°C) for 5 to 10 minutes. Centrifuge at 300 x g (≈ 2000 rpm) for 10 minutes and discard the supernatant. Add 1ml of DNA-XPress™ Reagent and mix the sample by repeated pipetting. Incubate for 5 minutes at room temperature (15-25°C).

7. **Liquid Matrices**

To isolate DNA from liquid matrices including stool, sputum, urine, wound exudates and viral cultures, gently homogenize 1ml sample in 10-15 ml of DNA-XPress™ Reagent.

8. **Mouse Tail**

Add pieces (1-3 mm) of mouse tail (up to 20 mg) to 0.5 ml Digestion Buffer supplemented with 400µg/ml Proteinase K. Incubate at 55°C for 1-4 hours with mixing or overnight at room temperature (15-25°C). Briefly centrifuge the samples and transfer the supernatant to a new tube. Add 0.5 ml of DNA-XPress™ Reagent to the supernatant and incubate at room temperature (15-25°C) for 5 minutes. Add 1ml of Ethanol (96-100%), mix and allow to stand for 1-3 minutes. Spool the DNA and proceed with Step E.

B. Phase Separation: (only for plants)

Add 1ml of chloroform per 1ml of DNA-XPress™ Reagent and allow the tube to stand for 5 minutes at room temperature (15-25°C). Centrifuge at 12,000 x g (≈ 13,000 rpm) for 10 minutes at room temperature (15-25°C). Transfer the upper aqueous phase to a clean tube. Precipitate the DNA with equal volumes of ethanol. Mix the sample by inverting the tube several times and allow it to stand for 5 minutes at room temperature (15-25°C). Sediment the precipitated DNA at 5000 x g (≈ 8,500 rpm) for 4 minutes and discard the resulting supernatant.

C. Centrifugation (Optional)

This step is **recommended for tissues** containing a large amount of extra cellular material (liver, muscle etc.) to remove any insoluble tissue fragments. Centrifuge at 10,000 x g (≈ 12,000 rpm) for 10 minutes at room temperature (15-25°C).

D. DNA Precipitation

Add 1ml of Ethanol (96-100%) per 1ml of DNA- XPress™ Reagent; mix the sample by inverting the tube several times. Allow the tube to stand for 3 minutes at room temperature (15-25°C). DNA should become visible. Remove the DNA by spooling with a pipette tip or centrifuge at 5000 x g (≈ 8,500 rpm) for 5 minutes. For small quantities of DNA use centrifugation.

E. DNA Wash

Wash the DNA pellet twice with 1ml of 95% ethanol. After the first wash with 1ml of 95% ethanol transfer the contents to a microcentrifuge tube and spin at maximum speed. Then remove the ethanol very carefully without leaving any residual ethanol in the tube.

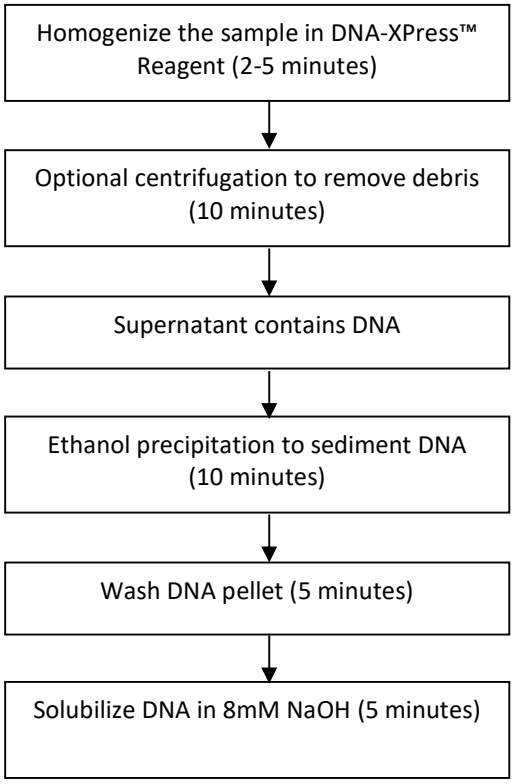
[From sources such as liver, kidney, yeast or gram positive bacteria, contaminants can be removed by washing with 50% DNA-XPress™ Reagent and 50% ethanol. Resuspend the DNA by inverting the tubes several times. Allow the DNA to settle to the bottom or centrifuge at 1,000 x g (≈ 3,000 rpm) for 1 minute. Remove the ethanol].

F. DNA Solubilization

Remove the remaining ethanol completely and air-dry the DNA pellet for 5 minutes. Do not let the DNA pellet to dry completely. Dissolve the DNA in sufficient amount of freshly prepared 8mM NaOH to reach the desired concentration.

NOTE: DNA concentration greater than 0.3µg/µl will cause a very viscous solution that will be difficult to work with. Store the sample at room temperature (15-25°C) for 5 minutes and dissolve the DNA by pipetting. For higher concentrations, heating at 55°C will be required. For preparation from tissues or plants containing insoluble material, remove the insoluble material by centrifugation at 12,000 x g (≈ 13,000 rpm) for 10 minutes.

Summary of the DNA- XPress™ Procedure



NOTE: The DNA obtained may contain some degraded RNA. If RNA-free DNA is required, add RNase A to the sample.

pH adjustment of DNA samples dissolved in 8mM NaOH

For 1ml of 8mM NaOH, use the following amounts of 1M HEPES (Product Code-MB016)

Final pH	1M HEPES (µl)
7.0	42
7.2	30
7.5	18
7.8	13.5
8.0	11.5
8.4	9.5

References

1. Chomczynski P., Mackey K., Drews R., Wilfinger W. 1997. DNAzol®: A reagent for the rapid isolation of genomic DNA. BioTechniques 22, 550-553.
2. Mackey K., Williams P., Seim S., Chomczynski P. 1996. The use of DNAzol® for the rapid isolation of genomic DNA from whole blood. Biomedical Products Supplement, 13-15.
3. Mackey K., Steinkamp A. and Chomczynski P. 1997. DNA Extraction from Small Blood Volumes and Single-Tube DNA Extraction and Amplification Using Blood Filter Cards. Molecular Biotechnology, 9, 1-5.
4. Chomczynski P., Wilfinger W. and Mackey K. 1998. Isolation of Genomic DNA from Human, Animal and Plant Samples with DNAzol® Reagents. Biotechnology International, 185-188.

Warning and Precautions

Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

1. The yield of DNA depends upon the type and the volume of starting material used.

Performance and Evaluation

Performance of the kit is expected when the kit is used as per the protocol mentioned in the product insert within the expiry period when stored at recommended temperature.

Quality Control

Type of Sample	DNA Yield	DNA Purity
1 ml whole blood	Upto 50 μg	1.6-1.9
50 mg plant sample	Upto 50 μg	1.6-1.9
50 mg animal tissue	Upto 50 μg	1.6-1.9

Troubleshooting Guide:

Sr. No.	Problem	Sample	Cause	Solution
1.	Low yield of DNA	Any sample	Incomplete homogenization of samples	No particulate matter should remain in the tube. Incubation for 5 minutes at room temperature (15-25°C) after homogenization is recommended.
			Insufficient volume of ethanol during precipitation	Add 1ml of Ethanol (96-100%) per 1ml of DNA-XPress Reagent.
			DNA pellet was not dissolved completely	Precipitate the DNA with ethanol and dissolve the pellet using a larger volume of 8mM NaOH. Heat the sample at 55°C for 10 minutes.
2.	Degraded DNA	Any sample	Samples were homogenized too vigorously, resulting in shearing of DNA	Avoid high-speed homogenizers and process DNA with adequate caution.
3.	RNA contamination	Any sample	Presence of RNA	Add RNase A at a final concentration of 0.2 mg/ml to the sample prior to the addition of ethanol, and incubate for 10 minutes at 37°C.

		Plant	The upper aqueous phase was removed with some of the organic phase	Remove the upper aqueous phase very carefully without the organic phase.
4.	Poor performance in downstream enzymatic applications.	Any sample	Residual reagents (ethanol, salts, etc.) present in prepared DNA	Precipitate DNA with ethanol, wash with cold 70% ethanol and dissolve in sterile molecular biology grade water.
5.	DNA does not dissolve in 8mM NaOH and forms a mucoid mass.	Any sample	Residual ethanol present in the tube	After the first wash with 95% ethanol transfer the DNA to a microcentrifuge tube and spin at high speed. Remove the ethanol very carefully without leaving any residual ethanol.
			The DNA obtained may be very concentrated	The amount of 8mM NaOH, Molecular Biology Grade Water or TE buffer to be added to the DNA for solubilization depends upon the concentration of DNA obtained which could be judged from the band intensity on an ethidium bromide stained agarose gel. Initially the amount of the solution to be added is around 100 µl which can be later increased to 500 µl in Molecular Biology Grade Water but till 400 µl in other solutions.

Safety Information

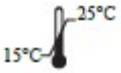
Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail to mb@himedialabs.com.



Storage temperature

Do not use if package is damaged

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