AuPreP DNA Extraction System

AuPreP DNA Extraction System is a high quality optimized system with ready-to-use reagent for quick isolation of total DNA from a large number of samples of different origin, viz human, animal, plant, yeast, bacterial and viral origin.

Cat. No. : AUP-DNAX-50

Storage : Room Temperature

AuPreP DNA Extraction System should be stored at room temperature. However, storing at lower temperatures will cause the guanidine isothiocyanate to come out of the solution. If the reagent is warmed, the guanidine isothiocyanate should resolubilize instantly.

System Details:

AuPreP DNA Extraction Kit is a non-organic and ready to use reagent for the isolation of genomic DNA from samples of human, animal, plant, yeast, bacterial and viral origin. It is based on disruption of cells in a guanidine-detergent lysing solution that hydrolyzes RNA and allows the selective precipitation of DNA from a cell lysate with ethanol. Following an ethanol wash, DNA is solubilized in water or 8 mM NaOH. There is no phenol in **AuPreP DNA Extraction System**. The protocol is fast and permits isolation of genomic DNA from a large number of samples of small or large volumes. The procedure can be completed in 10-30 minutes with DNA recovery of 70-100%. The isolated DNA can be used, without additional purification, for southern analysis, dot blot hybridization, molecular cloning, RFLP, PCR and other molecular biology and biotechnology applications.

System Components:

Solution containing 50 ml Guanidinium isothiocyanate.

Other products that may be required for your specific sample (not supplied with this system) includes:

- AuPreP Plant Solution (Cat. No. AUP-PLANT-10)
- AuPreP RBC Lysis Solution (Cat. No.AUP-RBC-LY-100)
- AuPreP Blood Solution (Cat. No. AUP-BLOOD-100)

Important Notes:

Please read the notes below before starting the procedure.

- *AuPreP DNA Extraction System contains irritants. Handle with care, avoid contact with skin, and use eye protection.*
- *In case of contact, wash skin with a large amount of water. Seek medical attention.*

Protocol for Genomic DNA Isolation

I	Homogenization
All samples should be gently, but thoroughly, homogenized with the AuPreP DNA Extraction System 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. Do not pipette the sample too vigorously, as this will shear the genomic DNA. All samples should be held at room temperature for 5 minutes, unless stated otherwise.	
a) Tissue	Gently homogenize tissue samples in the reagent. Use 1ml EZ-DNA per 50mg tissue.
b) Cells	**Cells grown in monolayer -** should be lysed directly in the culture dish by addition of 1ml AuPreP DNA Extraction System per $10cm^2$ area of culture dish. Discard the media, add AuPreP DNA Extraction System and pass the cell lysate several times through a pipette. **Cells grown in suspension -** use 1ml AuPreP DNA Extraction System per 10^7 cells. The cells should be pelleted and then lysed. Alternatively, use the suspension (volume < 0.1ml). **Cell nuclei -** use 1ml AuPreP DNA Extraction System per 10^7 cell nuclei. The nuclei can be either in pellet or suspension (volume < 0.1ml). Mix the samples by inverting the tubes or repeated pipetting.
c) Bacterial Cells	**Gram positive -** use 1ml AuPreP DNA Extraction System per 10^7cells. Freeze cells in liquid nitrogen and grind to a fine powder using a mortar and pestle, homogenize briefly, and gently mix for 1 hour at 60°C. **Gram negative -** sediment the cells and use 1ml AuPreP DNA Extraction System per 10^7 cells. Lyse the cells by repetitive pipetting and gently mix for 15-60 minutes at 60°C.
d) Yeast Cell	Sediment the cells and use 1ml AuPreP DNA Extraction System per 10^7cells. Homogenize briefly and gently mix for 15-60 minutes at 60°C.
e) Plant	Use 1ml AuPreP DNA Extraction System per 50-200mg of plant. Freeze cells in liquid nitrogen and grind to a fine powder using a mortar and pestle or homogenizer. Gently mix for 1 hr at 60°C, and proceed with section 2 of the protocol. For plant tissues containing polyphenolics, use **AuPreP Plant Solution (Cat. No. AUP-PLANT-10)** to assist with DNA isolation.
f) Liquid Matrices	To isolate DNA from liquid matrices including stool, sputum, urine, wound exudate and viral cultures, gently homogenize 1ml sample in 10-15ml AuPreP DNA Extraction System.
g) Whole Blood	To isolate DNA from fresh whole blood, add 1ml of whole blood to 2ml **AuPreP RBC Lysis Solution (Cat. No.AUP-RBC-LY-100).** Gently mix at room temperature for 5 to 10 minutes. Centrifuge at 300g for 10 minutes and discard the supernatant. Add 1ml AuPreP DNA Extraction System, and mix the sample by repeated pipetting. Hold for 5 minutes at room temperature. To isolate DNA from frozen blood, use **AuPreP Blood Solution (Cat. No. AUP-BLOOD-100)** for nuclei isolation prior to DNA extraction.
h) Mouse Tail	Add pieces (1-3mm) of mouse tail (up to 20mg) to 0.5ml digestion buffer supplemented with 400µg/ml Proteinase K. Incubate at 55°C for 1-4 hours with mixing, or overnight at room temperature. Briefly centrifuge the samples and transfer the supernatant to a new tube. Add 0.5ml AuPreP DNA Extraction System to the supernatant and incubate at room temperature for 5 minutes. Add 1ml of absolute ethanol, mix, and allow to sit for 1-3 minutes. Spool the DNA and proceed with Step V.
i) Biohazardous Material	When working with biohazardous material, Proteinase K digest can be used. This technique eliminates aerosols and improves biosafety.
II	**Phase Separation (PLANTS ONLY)**

Add 1ml chloroform per 1ml AuPreP DNA Extraction System. Allow to stand for 5 minutes at room temperature, and centrifuge at 12,000g for 10 minutes at room temperature. Following centrifugation, transfer the upper (aqueous) phase to a clean tube and precipitate the DNA by adding ethanol: 1 volume of aqueous phase with 1 volume of ethanol. Mix the samples by inverting the tubes 10 times and store them at room temperature for 5 minutes. Sediment precipitated DNA at 5,000g for 4 minutes and discards the resulting supernatant.

III Centrifugation (optional)`

This step removes insoluble tissue fragments, and is recommended for tissues containing a large amount of extracellular material (liver, muscle). Centrifuge at 10,000g for 10 minutes at room temperature.

IV DNA Precipitation

Add 1ml of absolute ethanol per 1ml of AuPreP DNA Extraction System. Mix samples by inverting the tubes 10 times. Make sure that the AuPreP DNA Extraction System and the ethanol make a homogenous solution. Store the samples for 3 minutes at room temperature. DNA should become visible. Remove the DNA by spooling with a pipette tip or centrifuge at 5,000g for 5 minutes. For small quantities of DNA use centrifugation.

V DNA Wash

Wash the DNA pellet twice with 1ml 95% ethanol. To remove contaminants from difficult sources (such as liver, kidney, yeast, gram positive bacteria), for the first wash use solution containing 50% AuPreP DNA Extraction System and 50% ethanol. Suspend the DNA by inverting the tubes 10 times. Allow the DNA to settle to the bottom or centrifuge at 1,000g for 1 minute. Remove the ethanol.`

VI DNA Solubilization

Remove remaining ethanol wash and air-dry the DNA pellet for 5 minutes. Do not let the DNA pellet dry completely. Dissolve the DNA in 8 mM NaOH (fresh preparation). Add a sufficient amount to reach your desired concentration. Note that a higher concentration than 0.3µg/µl will cause a very viscous solution that will be hard to work with. Store the sample for 5 minutes and then dissolve the DNA by pipetting. For high concentrations, heating at 55ºC will be required. For preparation from tissues or plants containing insoluble material, remove the insoluble material by centrifugation at 12,000g for 10 minutes. The final preparation of genomic DNA isolated with AuPreP DNA Extraction System contains 20-100 kb with the A_{260}/A_{280} at a ratio of 1.6-1.9.

Note

The resulting DNA may contain some degraded RNA. The concentration of the RNA is less than 3% of the DNA. For most methods this is no problem. If you require RNA-free DNA, apply RNase to the DNA sample.

pH Adjustment of DNA Samples Dissolved in 8mM NaOH

For 1ml of 8mM NaOH, use the following amounts of 1M Hepes, free acid:

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

Halting Points during Isolation

* The lysate which contains **AuPreP DNA Extraction System** can be stored for: 18 hours at room temperature
  9 months at 4°C
  9 months at -20°C
* During washes, DNA can be stored in 95% ethanol for at least 1 week at room temperature, or 3 months at 4°C.
* For long-term storage of high molecular DNA, re-precipitate the DNA and store in ethanol at 4°C.

Other AuPreP™ DNA/RNA Kits	Other Related Products
AuPreP™ Plasmid Maxi Kit **AuPreP™ Plasmid Midi Kit** **AuPreP™ SPIN^{m} SPIN Miniprep Kit** **AuPreP™ Blood Genomic DNA Maxi** **AuPreP™ Blood Genomic DNA Extraction Midi Kit** **AuPreP™ GEN^{bt} DNA Extraction Kit** **AuPreP™ DNA easy Plant Maxi kit** **AuPreP™ DNA easy Plant Mini Kit** **AuPreP™ PCR Purification Kit** **AuPreP™ Plant RNA Maxi Kit** **AuPreP™ Plasmid Maxi Kit** **AuPreP™ RNA Easy Midi Kit** **AuPreP™ RNA^{m} Mini Kit** **AuPreP™ RNV^{m} Viral RNA Extraction Miniprep Kit**	**AuPreP Oligos** (High Affinity Purified Oligo synthesis available in different scales, purifications & modifications) **AuPreP TaQ DNA Polymerase** (Ultrapure, Ultra-stable & Ultra-sensitive Taq DNA Polymerase) **AuPreP Hotstart TaQ DNA Polymerase** (Robust Polymerase for Hotstart PCR assays) **AuPreP Super Fidelity TaQ DNA Polymerase** (High fidelity Polymerase produces blunt ended amplicons upto 5Kb) **PCR Doctor** - (PCR enhancer for AuPreP Hotstart Taq or Super Fidelity Taq especially designed for GC/AT/Dirty/Difficult Templates **AuPreP Longjump Polymerase** (Robust Long Polymerase for templates > 4kb to 18kb+ for challenging PCRs) **AuPreP Red PCR Master Mix** (2x Master mix with Red Dye without Enhancer) **AuPreP DIAMOND MASTER-MIX** (2x Mastermix with PCR Enhancer & Stabilizer without tracking dyes) **AuPreP DIAMOND DOUBLE DYE MASTERMIX** (2x Mastermix with PCR Enhancer, Stabilizer & tracking dyes) **AuPreP DNA Extraction System** (A fast Reagent for pure genomic DNA isolation for down stream applications) **AuPreP RNA Extraction System** (for Purest & High Quality RNA extraction with simple cost effective protocol) **AuPreP Gold cDNA Synthesis Kit** (Highly Cost effective cDNA Synthesis Kit using RT with reduce Rnase H activity) **AuPreP Gold RT-PCR Combo Kit** (2 step RT-PCR protocol with tracking Dye) **AuPreP Extra Mile First Strand cDNA System** (Premium cDNA Synthesis Kit using RT with point mutant Rnase H minus activity) **Novascript III RNase H^{-} RT** (Premium Ultra-stable Rnase H minus RT for long high quality cDNA construction) **Novascript III single step RT-PCR System** (Premium 1step RT-PCR system using Novascript & AuPreP Hotstart DNA Polymerase) **AuPreP Random Primer labeling Mix System** (Premixed solution for the labeling of DNA with radiolabeled dCTP using random sequence oligonucleotides)

Reference

(1) Chomczynski, P. and Sacchi, N., *Anal Biochem.*, **162**:156-159 (1987)