AuPreP RNA Extraction System

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

Cat. No. : AUP-RNAX-100 **Storage: 2-8°C** **Shipping Conditions: 4°C**

System Details:

AuPreP RNA Extraction System is a complete system with ready-to-use reagents for the isolation of total RNA from samples of human, animal, plant, yeast, bacterial and viral origin. AuPreP RNA Extraction System is based on disruption of cells in guanidine thiocyanate/detergent solution, followed by organic extraction and alcohol precipitation of the RNA, and which allows simultaneous processing of a large number of samples. The resulting RNA is ultra pure and is suitable for the isolation of Poly A^+ RNA or for Northern Blotting, Dot Blotting, *in vitro* Translation, Molecular Cloning, RT-PCR and RNase Protection Assays, or other analytical procedures. DNA and proteins can also be recovered from the interphase and the organic Phase of same sample.

System Components:

1. **Solution A :** Denaturing Solution (Ultra Pure Grade Guanidine thiocyanate, 50ml).
2. **Solution B :** Extraction and Phase Separation Solution (Ultra Pure Grade Phenol and Chloroform,50ml)

Important Notes:

Please read the notes below before starting the procedure.

- *Use gloves and eye protection, avoid contact with skin or clothing, and avoid inhaling vapor because AuPreP RNA Extraction System contains phenol, which is poisonous, and guanidine thiocyanate, which is an irritant.*
- *In case of contact, wash immediately with plenty of water and seek medical advice.*

Protocol for RNA Isolation

Homogenization	**1. Tissue** Homogenize samples in the Denaturing Solution (0.5ml/50-100mg tissue) using homogenizer. Sample volume should not exceed 10% of the volume of the Denaturing Solution. **2. Cells** Cells grown in monolayer should be lysed directly in a culture dish using 0.5ml Denaturing Solution/10cm^2 of culture dish area. Pass the cell lysate several times through a pipette. Cells grown in suspension should be first sedimented, then lysed in the Denaturing Solution (0.5ml Denaturing Solution/5-10x10^6 for animal, plant or yeast cells; or 10^7 for bacterial cells) by repeated pipetting.
Phase Separation	Store the homogenate for 5 minutes at room temperature. Then add 0.5ml Extraction Solution per 0.5ml Denaturing Solution. Shake vigorously for 15 seconds, store at room temperature for 10 minutes and then centrifuge at 12,000g for 15 minutes at 4ºC.
RNA Precipitation	Transfer the aqueous colorless (upper) phase to a fresh tube and store the interphase and the organic phase at 4ºC for DNA isolation per 0.5ml Denaturing Solution. Store at room temperature for 10 minutes and then centrifuge at 12,000g for 8 minutes at 4ºC. ** To increase yield, perform second extraction: Transfer the upper phase and interphase to a fresh tube, add Extraction Solution of the above volume, and repeat centrifugation.*
LiCl Precipitation (optional)	Polysaccharides and other contaminants may be removed by by LiCl precipitation of the RNA. Re-suspend the RNA pellet by mixing with 2.5M LiCl solution. Vortex if necessary. Store at -20°C for at least 30 minutes and then centrifuge at 10,000g for 15 minutes at 4°C.
RNA Wash	Remove supernatant and wash the RNA pellet (by vortexing) with 1ml 75% ethanol. Then centrifuge at 7,500g for 5 minutes at 4°C. The RNA precipitate can be stored in 75% ethanol at 4°C for one week or at -20°C for at least one year.
RNA Solubilization	Remove the ethanol wash and air-dry the RNA pellet for 5 minutes. Do not let the RNA pellet dry completely. Dissolve the RNA in 100µl of DEPC-treated water with 0.1mM EDTA, or in 0.5% SDS solution (prepared with DEPC-treated water) by incubating for 10-15 minutes at 55°C. * Important: for best results in RT-PCR, dissolve the RNA in DEPC-treated water without EDTA (heat if necessary).
The final preparation of total RNA will be free of DNA and proteins, and will have a 260/280 O.D. ratio of 1.6 to 1.9.	

Protocol for DNA Isolation

DNA Precipitation	Carefully remove the remaining upper aqueous phase and discard. Add 0.3ml of absolute ethanol per 0.5ml of Denaturing Solution, and mix by inversion. Store at room temperature for 3 minutes and then centrifuge at 2000g for 5 minutes at 4°C. Remove the phenol-ethanol supernatant and store at 4°C for protein isolation (if desired).
DNA Wash	Wash the DNA pellet twice in a solution containing 0.1M Sodium Citrate in 10% ethanol. Use 1ml of solution per 0.5ml Denaturing Solution. Store at room temperature for 30 minutes with occasional mixing, and then centrifuge at 2,000g for 5 minutes at 4°C. Dissolve the DNA pellet in 75% ethanol (1.5-2ml per 0.5ml Denaturing Solution). Store at room temperature for 10-20 minutes with occasional mixing, and then centrifuge at 2000g for 5 minutes at 4°C.
DNA Solubilization	Remove the ethanol wash and air-dry for 5 minutes. Dissolve the DNA in 8mM NaOH by careful pipetting. Add 0.3-0.6ml 8mM NaOH to DNA isolated from 50mg of tissue or 107 cells. To remove any insoluble material, centrifuge at 12,000g for 10 minutes and transfer the supernatant to a new tube. Samples can be stored at 4°C overnight. For prolonged storage, adjust sample to pH 7-8 (with 1M Hepes, free acid) and adjust the EDTA concentration to 1mM.
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
Amplification of DNA by PCR	Following solubilization in 8mM NaOH, adjust the pH of the DNA sample to 8.4 with 1M Hepes, free acid. Add 0.1-1.0µg of the DNA sample to a PCR reaction mixture and perform the standard PCR protocol.
Digestion of DNA by Restriction Endonucleases	Adjust the pH of the DNA solution to a required value using 1M Hepes, free acid (see table).Use 3-5 units of enzymes per microgram of DNA. Use the conditions recommended by the enzyme manufacturer.

Protocol for Protein Isolation

Protein Precipitation	Precipitate proteins from the phenol-ethanol supernatant (step 2.1) with 1.5ml isopropanol per 0.5ml of Denaturing Solution used for the initial homogenization. Store samples for 10 minutes at room temperature and then centrifuge at 12,000g for 10 minutes at 4°C.
Protein Wash	Remove the supernatant and wash the pellet 3 times with 0.3M guanidine HCl in 95% ethanol. Use 2ml of wash solution per 0.5ml of Denaturing Solution for each wash. Store samples in wash solution for 20 minutes at room temperature. Centrifuge at 7,500g for 5 minutes at 4°C. After the final wash, add 2ml of absolute ethanol and vortex the protein pellet. Store for 20 minutes at room temperature and then centrifuge at 7500g for 5 minutes at 4°C.
Protein Solubilization	Air dry the protein pellet for 10 minutes. Dissolve the pellet in 1% SDS solution by pipetting. Complete solubilization of the protein pellet may require incubation at 50°C. Remove any insoluble material by centrifugation at 10,000g for 10 minutes at 4°C and transfer the supernatant to a new tube. The proteins may be used immediately for Western Blotting or stored at -20°C.

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

Last Updated on 04-04-2009

	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)