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AuPreP Citations

AuPreP™ GEL^X Gel Extraction Kit

Cat No. GX28-704LT
Cat No. GX28-706LT

Description

AuPreP™GEL^X Gel Extraction kit is designed to extract and purify DNA fragments from agarose gel. This kit is based on binding of up to 20 µg DNA to silica-based membranes in chaotropic salts with average recoveries of 60 to 90% of 100 bp to 10 kb DNA fragments.

Parameter	Value
Average preparation time	100 ~ 130 minutes
Workable length of fragment	1.5-kbp ~ 150-kb
Maximal recovery	99 %
Minimal elution volume	5ml
Maximal capacity	>100 µg
Regular sample volume	25 ml ~ 250 ml

Downstream Applications

* Restrictive enzymatic digestion
* Modifying enzymatic reaction
* Sequencing & PCR
* Ligation
* Labeling and Hybridization
* Radioactive and Fluorescent sequencing

Product Contents

	GX28-704LT (50 Preps.)	GX28-706LT (250 Preps.)
GEX Buffer	50 ml	250 ml
WN Buffer	6 ml*	30 ml**
WS Buffer	6 ml$^+$	30 ml^{++}
Elution Buffer	5ml	25 ml
Gel^X Column	50 pieces	250 pieces
Collection Tube	50 pieces	250 pieces
Protocol	1	1

*** For GX28-704LT**

- *Add 24 ml of 98-100% ethanol into WN and WS Buffer before first use. Please be sure to tighten the cap after each use when the ethanol has been added.*

****For GX28-706LT**

- Add 120ml of 98-100% ethanol into WN and WS Buffer before first use. Please be sure to tighten the cap after each use when the ethanol has been added.

Shipping and Storage

AuPreP™GEL^X Kit is stable at 20-25^{0}C for one year. Product should be stored in dry place and kept away from direct sunlight.

Important Notes

Please read the following notes before starting the procedures.

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1. Buffers provided in this kit contain irritants. Appropriate safety apparels such as gloves and lab coat, or even protective goggles should be worn. Peoples handling the kit may need suitable instruction.
2. All procedures should be done at room temperature (20-25^{0}C) and centrifugation should be done at full speed (10,000 x g or 13,000rpm) in a microcentrifuge, unless otherwise notified.
3. For long-term storage of the eluted DNA, TE buffer should be used for elution. Since EDTA in TE buffer may affect downstream applications, Elution Buffer (provided) or ddH_2O (pH 7.0 ~ 8.5) is preferred for the elution of DNA immediately used for downstream enzymatic reactions.
4. Please be aware that there are corresponding important notes listed below each step of the protocol. Important hints for user's references are listed beside the corresponding paragraph of the protocol. This information has been provided to help users minimize any potential problem.

AuPreP™ GEL^X Kit ……Protocol for Spin Method

	Related Notes
1. Use a clean, sharp scalpel or razor blade to excise the gel slice containing the DNA fragment of interest.	*Do NOT expose the gel to UV light for a long time as DNA will be nicked or denatured.* *Minimize the size of the gel slice by removing extra agarose.*
2. Measure the weight of the gel slice (about 50-200 mg) and place it into a sterile 1.5-ml or 2-ml centrifuge tube. Add 0.5 ml GEX Buffer into it.	*Cutting the gel slice into small pieces can facilitate dissolution.* *If more than 200 mg gel is used in order to harvest more DNA, increase the applied volume of GEX buffer proportionally.* *When agarose percentage of the gel slice is more than **2%**, adjust the use of GEX Buffer as 5x volumes of the gel slice (100 mg gel with 0.5ml GEX buffer).*
3. Incubate at 60^{0}C for 5 to 10 minutes until the gel is completely dissolved. Invert the tube every 1-2 minutes during incubation. Stop incubation when the gel has been completely dissolved. Let the gel mixture cool down to room temperature.	*Incubation with mixing can enhance gel dissolution.* *If gel dissolution cannot be completed in 10 minutes, refer to **Troubleshooting Guide** on page 12-13.* *Ensure that the gel has been completely dissolved before proceeding to step 4.*
4. Place a GEL^X Column onto a Collection Tube. Load no more than 0.7 ml dissolved gel mixture into the column.	*If the volume of dissolved mixture is more than 0.7ml, load and filter 0.7ml at each time until all the mixture has been filtrated.*
5. Centrifuge for 30-60 seconds. Discard the flow-through. Repeat step 4 for the rest of the mixture.	
6. Wash the column once with 0.5 ml of WN Buffer by centrifuging for 30-60 seconds. Discard the flow-through.	*Ensure that the ethanol has been added into WN buffer before first use.*
7. Wash the column once with 0.5 ml WS Buffer by centrifuging for 30-60 seconds. Discard the flow-through.	*Ensure that ethanol has been added into WS Buffer bottle when first open.*
8. Centrifuge the column at full speed for another 3 minutes to remove residual ethanol.	*Residual ethanol can affect the quality of DNA and inhibit subsequent enzymatic reactions. If necessary, centrifuging the column for a few minutes before eluting DNA.* *Do NOT remove ethanol by baking the column in an oven as high temperature may affect the intactness of the column.*
9. Place the column onto a new 1.5-ml centrifuge tube. Add 15-30 µl of Elution Buffer (provided) onto the center of the membrane.	*For effective elution, make sure that the elution solution is dispensed onto the **center** of the membrane and is **completely absorbed** (refer **to AuPreP™ GEL^x Hints**, No. 2 & 3, page 14).*
10. Stand the column for 3 minutes, and centrifuge at full speed for 1-2 minutes to elute DNA.	*If the solution still retains on the surface, pulse-centrifuging the tube for 1-2 seconds can drag the solution into the membrane. Do NOT over-centrifuge as the solution will get out of the membrane easily.*
11. Store DNA at -20^{0}C.	*Higher DNA recovery can be attained by eluting the column twice. That is, eluting twice with, e.g. 30µl H_2O or buffer, yields more DNA in total than eluting once with 60µl H_2O or buffer.*

AuPreP™ GEL ^{X} Kit …… Protocol for Vacuum Method

	Related Notes
1. Use a clean, sharp scalpel or razor blade to excise the gel slice containing the DNA fragment of interest.	*Do NOT expose the gel to UV light for a long time as DNA will be nicked or cleaved.* *Minimize the size of the gel slice by removing extra agarose.*
2. Measure the weight of the gel slice (about 50-200 mg) and place it into a sterile 1.5-ml or 2-ml centrifuge tube. Add 0.5 ml GEX Buffer to it.	*Cutting the gel slice into small pieces can facilitate dissolution. more than 200 mg gel is used to harvest more DNA, increase the applied volume of GEX buffer proportionally.* *When agarose percentage of the gel slice is more than 2%, add GEX Buffer as 5x volumes of the gel slice (100 mg gel with 0.5ml GEX buffer).*
3. Incubate at 60^{0}C for 5 to 10 minutes until the gel is completely dissolved. Invert the tube every 1-2 minutes during incubation. Stop incubation when the gel has been completely dissolved. Let the gel mixture cool down to room temperature.	*If gel dissolution cannot be completed in 10 minutes, refer to* ***Troubleshooting Guide*** *on page 12-13.* *Ensure that the gel has been completely dissolved before proceeding to step 4. Gently revert the tube, and observe the contents in vial with back light, to see if there is any gel-like substance remaining.*
4. Insert a GEL ^{X} Column into the luer-lock of a vacuum manifold (e.g. Promega's Vac-man*). Load no more than 0.7 ml of the dissolved gel mixture into the column.	*If the volume of dissolved mixture is more than 0.7ml, load and filter 0.7ml at each time until all the mixture has been filtrated.*
5. Apply vacuum to draw all the liquid into the manifold. Load the rest of the mixture.	
6. Wash the column once with 0.5 ml of WN Buffer by re-applying vacuum to draw all the liquid.	*Ensure that the ethanol has been added into WN and WS buffer before first use.*
7. Wash the column once with 0.5 ml WS Buffer by re-applying vacuum to draw all the liquid.	*Keep the cap of the WN and WS bottle tight after each use to avoid volatilization of ethanol. Decreased ethanol content in WN or WS buffer may cause DNA loss during wash.*
8. Place the column onto a Collection Tube. Centrifuge the column at full speed for 3 minutes to remove residual ethanol.	*Residual ethanol can affect the quality of DNA and inhibit subsequent enzymatic reactions. If necessary, centrifuging the column for a few minutes more can remove all the ethanol before eluting DNA.* *Do NOT remove ethanol by baking the column into an oven as high temperature may affect the intactness of the column.*
9. Place the column onto a new 1.5-ml centrifuge tube. Add 15-30 µl of Elution Buffer (provided) onto the center of the membrane.	*For effective elution, make sure that the elution solution is dispensed onto the center of the membrane and is completely absorbed (refer to AuPreP™ GEL^{X} Hints, No. 2 & 3, page 14).*
10. Stand the column for 3 minutes, and centrifuge for 1-2 minutes to elute DNA.	*If the solution still retains on the surface, pulse-centrifuging the tube for 1-2 seconds can drag the solution into the membrane.* *Do NOT over-centrifuge as the solution will get out of the membrane easily.*
11. Store DNA at -20^{0}C.	*Higher DNA recovery can be attained by eluting the column twice. That is, eluting twice with, e.g. 30 µl H_2O or buffer, yields more DNA in total than eluting once with 60 µg H_2O or buffer.*

*Vac-man is a trademark of Promega Corporation

AuPreP™ GEL ^{X} Kit …… Troubleshooting Guide

Problem	Possible Reason	Solution
Gel slice hard to dissolve	Used High percentage agarose gel	*When the agarose percentage is >2.0%, and GEX Buffer at 5 times the volume of the gel slice (100 mg= 0.5ml). When the agarose percentage of the gel is > 2.5%, add GEX Buffer at 6 times the volume of the gel slice (100mg = 0.6 ml). Mix every 1-2minutes during the incubation until complete dissolution.*
	Gel slice is too big (more than 200 mg)	*Use more than one column for gel slice more than 200 mg.*

Problem	Possible Reason	Solution
Low recovery of DNA fragment	Incomplete dissolution of the gel slice	*Check the dissolution mixture with a back light to see if there is any gel-like substance remained.*
	Ineffective DNA elution	*DNA elution does not take place well at acidic conditions. Make sure that ddH_2O used is of pH between 7.0 and 8.5.*
	Incomplete DNA elution	*Complete DNA elution only takes place when elution solution is in full contact with the membrane. Make sure that no less than 15 μl of solution is dispensed onto the membrane and is completely absorbed into it before centrifugation.*
	TAE or TBE buffer is repeatedly used for many times or of incorrect pH	*pH of repeatedly used TAE or TBE buffer usually gets increased. Use fresh TAE or TBE buffer each time.*
	Overload the column with too much agarose	***Higher*** *recovery is attained when lower amount of agarose gel is present. Minimize the size of the gel slice by removing extra gel. When gel slice is more than 200 mg, use more than one column.*
	Size of DNA fragment is more than 5 kb	*Use elution solution preheated to 60^{0}C*
Poor Performance in downstream applications	Eluted DNA carries salt residue	*Wash the column twice with 0.5 ml WS Buffer.*
	Eluted DNA carries ethanol residue	*After wash with WS Buffer, do discard the flow-through, and centrifuge the column for another 3 minutes. If necessary, centrifugation for a few minutes more can completely remove ethanol. However, do* ***not*** *remove ethanol by putting the column into an oven as high temperature may affect the intactness of the column.*
Non-specific DNA fragment appears in eluted DNA	DNA fragment is denatured and becomes single-stranded	*To re-anneal the single-stranded DNA, incubate the tube at 95^{0}C for 2 minutes and let it cool slowly to room temperature. Re-annealed DNA fragments are applicable for all downstream applications.*
	Scalpel or razor blade used to excise the gel is contaminated with other DNA fragments	*Use a new or clean scalpel or razor blade to excise the gel.*
Poor OD_{200}/OD_{280} radio	Use of H_2O of acidic pH to dilute the eluted DNA	*Make sure the H_2O has the pH value between 7.0-8.5*

AuPreP™ GEL^X Kit …… Hints

1. Milli-Q or double-distilled H_2O stored in a laboratory for a period of time usually becomes acidic due to dissolution of CO_2 or other acidic vapor such as HCl from air. Always check the pH to make sure that it is between 7.0 to 8.5 before used. Use H_2O of pH less 7.0 for elution will lead to reduced yield of DNA. Use H_2O of acidic pH (pH 5.0-6.0) to dilute DNA or RNA samples for spectrophotometric analysis will also significantly decrease A_{260}/A_{280} ratio of the sample (Wilfinger et al., 1997).

2. Higher DNA recovery can be attained by eluting the column twice. That is, eluting twice, e.g., with 30 μl H_2O or buffer, yields more DNA in total than eluting once with 60 μl H_2O or buffer.

3. Use of elution solution preheated to 60 ^{0}C can increase recovery of DNA fragment larger than 5 kb.

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** (Premixed solution for the labeling of DNA with radiolabeled dCTP using random sequence oligonucleotides)

Reference: Wilfinger, W. W., Mackey, K., and Chomczyski, P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *Biotechniques* **22**:474-481

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