



See Some Important Members of
AuPreP Family

AuPreP Citations

AuPreP™ GEN^{bt} DNA Extraction Kit

Cat. No. GEN51-304LT

Cat. No. GEN51-306LT

Description with Protocol

AuPreP™ GEN^{bt} DNA Extraction Kit provides a fast and efficient method to purify genomic DNA from various samples such as cultured animal cells, animal tissues, whole blood, buffy coat, lymphocytes, plasma, serum, bacteria, yeasts, DNA virus, paraffin-embedded tissues, etc. Without need of time-consuming phenol/chloroform extraction and ethanol precipitation, this simple spin-column method can isolate genomic DNA of predominantly 20-30 kb free from protein and salt contaminants.

Sample	Maximum Amount	Yield(µg)	Preparation Time
Whole Blood	200µl	Up to 10*	50 min
Animal Cells	10 ⁷	Up to 100	50 min
Animal Tissue	30mg	Up to 100*	1.5hr
Mouse Tail	0.5cm	Up to 15	1.5-5 hr
Bacteria/Yeast	10 ⁹ / 10 ⁸	Up to 80	1.5 hr
Paraffin-Embedded tissue	25mg(including paraffin)	Up to 100*	2 hr

* Yield depends on individuals or types.

Downstream Applications

- * PCR
- * Restriction Analysis
- * Southern Blotting

Product Contents

	50 Preps	250 Preps
LYS Buffer	12 ml	60 ml
EX Buffer	13ml	60 ml
WS Buffer	15ml*	45 ml*
Proteinase K	10mg**	10mg x 5**
B/T Genomic DNA Mini Column	50 pieces	250 pieces
Collection Tube	100 pieces	500 pieces
Protocol	1	1

* For (50 preps), add 60ml of 98-100% ethanol into WS Buffer bottle when first open.

* For (250 preps), add 180ml of 98-100% ethanol into WS Buffer bottle when first open.

** Add 1ml sterile ddH₂O to reconstitute one tube of the provided Proteinase K by vortexing for 1 minute. Make sure that Proteinase K has been completely dissolved. The solution should look clear. The concentration of the Proteinase K stock solution is 10 mg/ml. Store the solution at 4°C.

Shipping and Storage

All components of AuPreP™ GEN^{bt} DNA Extraction Kit are stable at room temperature (20-25°C) for one year.

Important Notes

Please read the following notes before starting the procedures

1. Buffers in this system contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn to protect from skin contact.
2. All procedure should be done at room temperature (20-25⁰C).
3. Prepare a 60⁰C and/or 70⁰C water bath or incubator
4. Add 1 ml sterile ddH₂O to reconstitute the provided Proteinase K by vortexing. Make sure that Proteinase K has been completely dissolved. The solution should look clear. The concentration of the Proteinase K stock solution is 10 mg/ml. Store the solution at 4⁰C.
5. Do **not** add and keep Proteinase K directly in EX Buffer.
6. For 50 preps, add **60 ml** of 98-100% ethanol into each WS Buffer bottle **when first open**. For (250 preps), add **180 ml** of 98-100% ethanol into WS Buffer bottle when first open. Ethanol is provided by the user
7. Centrifuge steps done at full speed refers to 10,000 x g or 13,000-14,000 rpm of a microcentrifuge
8. Do **not** use more than the suggested maximum amount of sample (refer to **AuPreP™ GEN^{bt} Hints**, No. 1, page 21)
9. Homogenization of the tissue sample can greatly **reduce** the time of sample lysis.
10. RNA may be copurified with genomic DNA. RNA will not affect PCR, but may affect certain downstream applications. If RNA-free genomic DNA is desired, add RNase A to the sample as indicated in the protocol.
11. After each vortexing step, when the tube is opened, **briefly centrifuge** the tube to bring down the sample attached inside the cap to avoid generation of aerosols and contact with sample.
12. When sample or buffer is added into the column, avoid touching the rim. This is to prevent cross contamination of samples when handling the columns.
13. DNA can be eluted in 10 mM Tris-HCl (pH 9.0), Milli-Q or double-distilled H₂O, or TE buffer (pH 8.0). Since genomic DNA elution takes place most effectively at **pH 9**, to ensure optimal elution, make sure that pH of these elution solutions are between 8.0 and 9.0.

Protocol

Please refer to the Table of Contents on page 4 to choose the appropriate protocol according to the kind of sample used.

I. Blood Protocol

For samples including whole blood (anti-coagulant added), buffy coat, serum, plasma, body fluid, 10⁶-10⁷ lymphocytes and cultured cells in 200µl PBS.

	<i>Related Notes</i>
1. Pipet up to 200 µl sample into a 1.5-ml sterile eppendorf tube. When the sample volume is less than 200µl, add PBS to make up to 200 µl.	<i>If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the sample at this step.</i>
2. Add 20 µl Proteinase K and 200 µl EX Buffer into the sample. Mix immediately by vortexing for 20 seconds.	<i>Do NOT add and keep Proteinase K directly in EX Buffer.</i> <i>When sample volume is larger than 200µl, increase the amount of Proteinase K and EX Buffer proportionally.</i>
3. Incubate at 60 ⁰ C for 20 minutes to lyse the sample. Vortex or invert mix the sample every 3-5 minutes during incubation.	<i>Ensure complete sample lysis:</i> <i>whole blood sample should NOT appear viscous;</i> <i>buffy coat should NOT contain insoluble residues;</i> <i>cell sample should appear translucent.</i>
4. Adjust the incubator to 70 ⁰ C to incubate for 20 minutes.	<i>Alternatively, place the sample to another 70⁰C incubator and incubate for 10 minutes.</i>
5. Meanwhile, preheat 10 mM Tris-HCl (pH 9.0), ddH ₂ O, or TE buffer (provided by user) at 70 ⁰ C (500 µl /prep) for DNA elution at Step 10.	<i>Refer to Important Notes, No. 13, page 8, and AuPreP™ GEN^{bt} Hints, No. 3, page 22, for the choice of elution solution.</i>
6. Add 210 µl of absolute ethanol or isopropanol to the sample from Step 4 and mix by vortexing.	<i>If the sample volume is more than 200µl, increase the amount of ethanol or isopropanol proportionally</i>
7. Place a B/T Genomic DNA Mini Column onto a Collection Tube. Pipette all the mixture (including any precipitate) into the column	<i>If a precipitate formed in Step 6, apply both the precipitate and mixture into the column.</i>

without touching the rim. Centrifuge at 8,000 rpm (6,000 x g) for 2 minutes. Place the column onto a new Collection Tube.	
8. Wash the column twice with 0.5 ml WS Buffer by centrifuging at 8,000 rpm (6,000 x g) for 2 minutes. Discard the flow-through after centrifugation.	<i>Ensure that ethanol has been added into WS Buffer bottle when first open.</i>
9. Centrifuge the column at full speed for another 2 minutes to remove ethanol residue	<i>Refer to Important Notes, No. 7, page 7.</i>
10. Place the column onto a new 1.5-ml tube(provided by user).Elute DNA with 200 µl of the preheated elution solution from Step 5.	<i>Refer to AuPrep™ GEN^{bt} Hints, No. 5, page 22, for optimal DNA elution.</i>
11. Stand the column for 1-5 minutes, and centrifuge for 1-2 minutes to elute DNA.	
12. Store eluted DNA at 4°C or -20°C	<i>Store DNA at 4°C for frequent use or at -20°C for long-term storage. Repeated freeze-thaw cycles can cause shearing of genomic DNA.</i>

II Tissue Protocol

	<i>Related Notes</i>
1. Cut 30 mg of tissue (15 mg spleen) into small pieces and place the sample into a 1.5-ml sterile eppendorf tube. Add 200 µl LYS Buffer and homogenize the sample.	<i>If the sample size is larger than 30 mg, increase the amount of LYS Buffer proportionally.</i>
2. Add 20 µl Proteinase K to the sample. Mix immediately by vortexing for 20 seconds.	<i>If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml Rnase A to the sample.</i>
3. Incubate at 60°C for 1 hour to lyse the sample. If tissue is difficult to lyse, increase the incubation time to 2-3 hours. Vortex or invert mix the sample every 10-15 minutes .	<i>Vortex-mixing is important for complete breaking up and digesting of the tissue. Ensure complete sample lysis; sample after complete lysis should appear translucent.</i>
4. Adjust the incubator to 70°C to incubate for 20 minutes.	<i>Alternatively, place the sample to another 70°C incubator and incubate for 10 minutes.</i>
5. Meanwhile, preheat 10 mM Tris-HCl (pH 9.0), ddH ₂ O, or TE buffer (provided by user) at 70°C (500 µl /prep) for DNA elution at Step 11.	<i>Refer to Important Notes, No. 13, page 8, and AuPreP™ GEN^{bt} Hints, No. 3, page 22, for the choice of elution solution.</i>
6. Add 200 µl of EX Buffer to the sample, mix by vortexing and incubate at 70°C for 10 minutes.	<i>If the sample contains undigested remains after incubation, centrifuge for 5 minutes at full speed and use only the supernatant in the following steps.</i>
7. Add 210 µl of absolute ethanol or isopropanol to the sample and mix by vortexing.	<i>If the sample mixture is more than 400µl, increase the amount of ethanol or isopropanol proportionally.</i>
8. Place a B/T Genomic DNA Mini Column onto a Collection Tube. Pipette all the mixture (including any precipitate) into the column without touching the rim. Centrifuge at 8,000 rpm (6,000 x g) for 2 minutes. Place the column onto a new Collection Tube.	<i>If a precipitate formed in Step 7, apply both the precipitate and mixture into the column.</i>
9. Wash the column twice with 0.5 ml WS Buffer by centrifuging at 8,000 rpm (6,000 x g) for 2 minutes. Discard the flow-through after centrifugation.	<i>Ensure that ethanol has been added into WS Buffer bottle when first open.</i>
10. Centrifuge the column at full speed for another 2 minutes to remove ethanol residue.	<i>Refer to Important Notes, No. 7, page 7.</i>
11. Place the column onto a new 1.5-ml tube (provided by user). Elute DNA with 200 µl of	<i>Refer to AuPreP™ GEN^{bt} Hints, No. 5, page 22,for optimal DNA elution.</i>

the preheated elution solution from Step 5	
12. Stand the column for 1-5 minutes, and centrifuge for 1-2 minutes to elute DNA	
13. Store eluted DNA at 4 ⁰ C or -20 ⁰ C	<i>Store DNA at 4⁰C for frequent use or at -20⁰C for long-term storage. Repeated freeze-thaw cycles can cause shearing of genomic DNA.</i>

III. Mouse Tail Protocol

	<i>Related Notes</i>
1. Cut into small pieces of a segment of mouse tail of up to 0.5 cm. Place the sample into a 1.5-ml sterile tube.	<i>Segment close to the tail tip is preferred. Segment away from the tip is thicker and takes longer time to lyse completely.</i>
2. Add 20 µl Proteinase K and 200 µl LYS Buffer to the sample. Mix immediately by vortexing for 20 seconds.	<i>If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the sample. Further addition of 20 µl of 10 mg/ml Proteinase E (Dnase-free) (provided by user) can enhance mouse tail lysis and increase DNA yield.</i>
3. Incubate at 60 ⁰ C for 1-4 hours or overnight to lyse the tail tissue. Vortex or invert mix the sample every 20-30 minutes during incubation.	<i>Ensure complete sample lysis; sample after complete lysis should appear translucent with only hair and bone residues remained.</i>
4. Follow the Tissue Protocol starting from Step 4 on Page 11.	

IV. Paraffin-Embedded Tissue Protocol

1. Cut a small section of paraffin-embedded tissue (about 25 mg) and put into a 1.5-ml eppendorf tube.	
2. Add 1 ml xylene and incubate at room temperature with occasional mixing for 30 minutes to extract paraffin from tissue	
3. Centrifuge at full speed for 5 minutes. Remove the supernatant by pipetting.	<i>Refer to Important Notes, No. 7, page 7.</i>
4. Add 1 ml absolute ethanol to the tissue pellet, mix, and centrifuge at full speed for 5 minutes. Remove ethanol- containing xylene residue by pipetting.	
5. Evaporate ethanol residue by incubating at 37 ⁰ C for 10 minutes.	
6. Resuspend the pellet in 200 µl LYS Buffer	
7. Follow the Tissue Protocol starting from Step 2 on page 11.	

V. Bacteria Protocol.

A. Bacteria

1. Pellet log-phase grown bacteria of up to 10 ⁹ (or up to 3 ml culture) at 7,500 rpm (5,000 x g) for 10 minutes.	
--	--

2. Resuspend the pellet in 200 µl lysozyme reaction solution (20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 20 mg/ml lysozyme). Incubate at 37°C for 30 minutes.	If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml Rnase A to the resuspended cells. Lysozyme is provided by user.
3. Add 20 µl Proteinase K and 200 µl EX Buffer to the sample. Mix immediately by vortexing for 20 seconds.	Do NOT keep Proteinase K directly in EX Buffer.
4. Incubate at 60°C for 30 minutes to lyse the bacterial cells. Vortex or invert mix the sample every 5 minutes during incubation.	Incubation with mixing facilitates lysis. Ensure complete cell lysis; sample after complete lysis should appear translucent.
5. Adjust the incubator to 70°C to incubate for 30 minutes.	
6. Follow the Blood Protocol starting from Step 5 on Page 9.	

B. Bacteria in biological fluids

1. Pellet cells by centrifuging at 7,500 rpm (5,000 x g) for 10 minutes.	
2. Resuspend the pellet in 200 µl LYS Buffer.	If RNA-free genomic DNA is desired, add 5 µl of 50 mg/ml Rnase A to the resuspended cells.
3. Follow the Tissue Protocol starting from Step 2 on page 11.	

C. Bacteria from eye, nasal or pharyngeal swabs

1. Collect bacterial cells by rinsing and soaking the swabs in 2 ml PBS at room temperature for 2-3 hours.	
2. Pellet cells by centrifuging at 7,500 rpm (5,000 x g) for 10 minutes.	
3. Resuspend the pellet in 200 µl LYS Buffer.	If RNA-free genomic DNA is desired, add 5 µl of 50 mg/ml Rnase A to the resuspended cells.
4. Follow the Tissue Protocol starting from Step 2 on page 11.	

VI. Yeast Protocol

1. Pellet log-phase grown yeast cells up to 10 ⁸ (or up to 3 ml culture) at 7,500 rpm (5,000 x g) for 10 minutes.	
2. Resuspend the pellet in 500 µl sorbitol reaction solution (1 M sorbitol; 100 mM EDTA; 14 mM β-mercaptoethanol; 200 U lyticase or zymolase).	If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the resuspended cells. Lyticase or zymolase is provided by user.
3. Incubate at 30°C for 30 minutes.	
4. Centrifuge at 7,500 rpm (5,000 x g) for 5 minutes. Resuspend the pellet in 200 µl LYS Buffer.	
5. Follow the Tissue Protocol starting from Step 2 on page 11.	

VII. Virus Protocol

1. Prepare viral DNA from blood or body fluid , the Blood Protocol on page 9-10 is suggested.
2. Prepare integrated viral DNA , the Blood Protocol on page 9-10 or Tissue Protocol on page 11-12 is suggested.

Troubleshooting Guide

Problem	Possible Reason	Solution
Brown color residues remain on the membrane of a column after washing	Incomplete digestion of hemoglobin	Vortex the sample after Proteinase K is added. Mix the sample every 3-5 minutes during incubation.
	No alcohol or alcohol of incorrect amount is added to the sample before loaded into the column	Before passing the column, add 210 µl (or suitable volume) of absolute alcohol into the sample.
	WS Buffer does not contain ethanol	Make sure that ethanol is added into the WS Buffer bottle when first open (refer to Important Notes , No. 6, page 7).
Low or no yield of DNA	Sample contains too low amount of genomic DNA	Increase the sample amount, Proteinase K, and buffer proportionally. If the sample is whole blood, prepare buffy coat from a larger volume of blood.
	Blood or cell sample is not lysed completely	Add another 20 µl fresh Proteinase K per sample and repeat incubation.
	No alcohol or alcohol of incorrect amount is added to the sample before loaded into the column	Before passing the column, add 210 µl (or suitable volume) of absolute alcohol to the sample.
Low or no yield of DNA	Elution solution is not preheated at 70°C	Preheat the elution solution at 70°C before used.
	pH of the elution solution is too low	Make sure that the pH of 10 mM Tris-HCl, ddH ₂ O or TE buffer for elution is between 8.0-9.0.
	WS Buffer does not contain ethanol	Make sure that ethanol is added into the WS Buffer bottle when first open (refer to Important Notes , No. 6, page 7).
Column is clogged when passing the sample	Tissue sample still contains undigested remains after lysis	After Proteinase K digestion, centrifuge the sample at full speed for 5 minutes to remove undigested remains.
	Blood sample contains clots	Use whole blood sample mixed well with anti-coagulant to prevent formation of blood clot. Do not use blood clot for genomic DNA extraction.
	Sample is very viscous	Too much sample is used. Reduce the sample amount.
A ₂₆₀ /A ₂₈₀ ratio of eluted genomic DNA is low	Protein in the sample is not completely degraded	Vortex the sample after Proteinase K is added. Mix the sample at constant intervals during incubation.
	Protein in the sample is not completely degraded	Add 20 µl fresh Proteinase K per sample and continue incubation.
	No alcohol or alcohol of incorrect amount is added to the sample before loading into the column	Before passing the column, add 210 µl (or suitable volume) of absolute alcohol into the sample.
	Eluted genomic DNA contains contaminants	Do not touch the rim of the column during sample or buffer loading.
	Eluted genomic DNA contains ethanol	After the final wash, centrifuge the column at full speed for another 2 minutes to remove the ethanol residue completely.
	Using ddH ₂ O of acidic pH (5.0-6.0) to dilute DNA samples for spectrophotometric analysis	Use 10 mM Tris-HCl of pH 7.5 or TE buffer to dilute the DNA samples (refer to AuPrep™'s GEN^{ht} Hints , No. 4, page 22).
A ₂₆₀ /A ₂₈₀ of eluted genomic DNA is high (>1.9)	Eluted genomic DNA contains a lot of RNA	Add RNase A to the sample as described in the protocol.
Genomic DNA appears smearing and degraded	Sample is not fresh or stored improperly for a long time	Flash freeze fresh samples in liquid nitrogen and store at -80°C if not used immediately.
	Blood sample is not fresh or stored improperly for a long time	Use fresh blood, or blood stored at room temperature for fewer than 2 days.
	Gel electrophoresis is performed in used running buffer contaminated with Dnase	Use fresh TAE or TBE running buffer for electrophoresis.
	Paraffin-embedded tissue is used as sample	Genomic DNA isolated from this kind of sample is usually degraded. It is still suitable for PCR application, but is not recommended for Southern blotting and restriction analysis.

AuPreP™'s GEN^{bt} DNA Extraction Kit Hints

1. Low yield or purity of genomic DNA is usually due to incomplete digestion or lysis of the sample. Starting with a maximum amount or volume of samples does NOT usually give the best yield of DNA. Instead, it always results in incomplete sample lysis and degradation of proteins, thus making extraction of all DNA from the sample unfeasible. Further, it always requires subsequent removal of undigested residues and yields viscous sample lysate. When the lysate is too viscous, it not only has difficulty in passing the column, but also indicates the presence of an abundant amount of contaminants such as proteins and salts. Contaminants of high amount not only affect DNA binding, but also may not be washed off completely, leading to carry over to the eluted genomic DNA. Therefore, a good quality and yield of DNA is only expected when a sample is **completely** digested. We advise starting with half of the maximum amount of sample suggested. When there is no problem in digesting the sample completely and passing the lysate through the column, amount of the sample to be applied can be increased gradually in the subsequent preparations.
2. When buffy coat is used as sample, make sure that cells in it are not more than 1×10^7 , otherwise the lysate obtained will be too viscous to pass through the column easily.
3. DNA should not be eluted in ddH₂O for storage because it suffers from gradual degradation through acid hydrolysis. Since DNA is more stable in a slightly alkaline (pH 7.5-9) buffering condition, 10 mM Tris-HCl (pH 9.0) or TE buffer is considered a better choice than water for DNA elution. When TE buffer is used for elution, make sure that EDTA in the buffer does not affect further enzymatic reaction.
4. Using ddH₂O of acidic pH (5.0-6.0) to dilute DNA and RNA samples for spectrophotometric analysis will significantly decrease A₂₆₀/A₂₈₀ ratio of the sample (Wilfinger et al., 1997) 10 mM Tris-HCl of pH 7.5 or TE buffer should be used to dilute the samples.
5. Elute DNA according to the yield expected. Use 50 µl elution solution for less than 1 µg DNA, 100 µl for less than 5 µg DNA, and 200 µl twice for more than 30 µg DNA. Generally, a higher DNA recovery can be attained by eluting the column twice. That is, eluting twice, with e.g. 100 µl elution solution, yields more DNA in total than eluting once with 200 µl elution solution.

<u>Other AuPreP™ DNA/RNA Kits</u>	<u>Other Related Products</u>
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™ 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™ 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)
	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 Chomczynski, P. 1997. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *Biotechniques* **22**:474-481.