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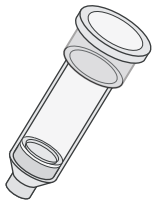
GenCatch™ Genomic DNA Extraction Kit



User's Guide for

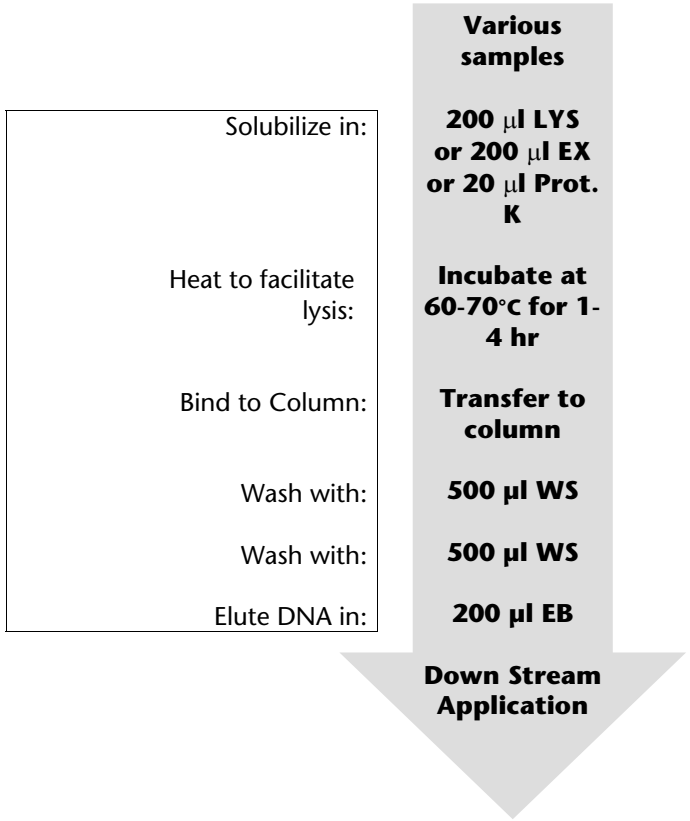
Genomic DNA Purification from Blood, Tissue, Bacteria, Yeast and Virus

For Research Use Only

1 Quick Start Procedure

This Quick Start Procedure Is For experienced users only.
First time users are strongly recommended to read through the detailed protocol in section 4.

Before you start:
*Add **60** ml (50 preps) or **180** ml (250 preps) 98-100% ethanol to WS Buffer.*



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2 Overview

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

Preparation time: 1-4 hr depends on sample sources

Downstream Applications:

- Southern blotting
- Restriction enzyme digestion
- Genomic library construction
- PCR
- Genotyping

	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.

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Product Contents

GenCatch™ Blood/Tissue Genomic DNA Kit contains sufficient reagents for 50 (Cat. No. 1460050) and 250 (Cat. No. 1460250) genomic DNA purifications respectively.

Catalog Number	1460050	1460250
LYS Buffer	12 ml	60 ml
EX Buffer	13 ml	60 ml
WS Buffer	15 ml	45 ml x2
Proteinase K	10 mg	10 mg x5
GenCatch™ Column	50 pieces	250 pieces
Collection Tube	100 pieces	500 pieces
Protocol	1	1

Add 60 ml (50 preps) or 180 ml (250 preps) 98-100% ethanol to WS buffer bottle when first opened.

Add 1 ml sterile ddH$_2$O 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 is 10 mg/ml. Store the solution at 4°C.

Storage Conditions:
Store at room temperature

All components are guaranteed for 48 months from the date of purchase, when stored under specified conditions and used as described in this manual. ***GenCatch™*** Column has no definite expiration date as long as it is kept away from contamination.

4 Protocol

First time users are strongly recommended to read through this detailed protocol instruction.
For technical support and user raised common questions and answers please visit: ***www.epochbiolabs.com***

Before you start:
Add 60 ml (50 preps) or 180 ml (250 preps) 98-100% ethanol to Buffer WS before use (see instructions on bottle label).

I. Blood Protocol:

For samples including whole blood (anti-coagulant added), buffy coat, serum, plasma, body fluid, 10^6-10^7 lymphocytes and cultured cells in 200 µl PBS.

1. **Pipet up to 200 µl sample into a 1.5 ml sterile eppendorf tube. Use PBS to make up to 200 µl if volume is less than 200 µl.**
   If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the sample at this step.

2. **Add 20 µl Proteinase K and 200 µl EX Buffer into the sample. Mix immediately by vortexing for 20 seconds.**
   Do Not add and keep Prot. K directly in EX Buffer. When sample volume > 200 µl, increase the amount of Prot. K and EX Buffer proportionally.

3. **Incubate at 60°C for 20 minutes to lyse the sample. Vortex or invert mix the sample every 3-5 minutes during incubation.**
   Ensure complete sample lysis, sample should appear translucent.

4. **Adjust the temperature to 70°C and incubate for another 20 minutes.**

	before loaded into the column	sample.
	Eluted genomic DNA contains contaminants	Do not touch the rim of the column during sample or buffer loading.
	Eluted genomic DNA carries ethanol	After the final wash, centrifuge the column at full speed for another 2 minutes to remove the ethanol residue completely.
	Using ddH_2O of acidic pH (5.0-6.0) to dilute DNA sample for spectrophotometric analysis	Use 10 mM Tris-HCl of pH 7.5 or TE buffer to dilute the DNA sample.
A260/A280 ratio 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 sample 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.

	column	
	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_2O 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.
Column is clogged when passing the sample	Tissue sample still remains undigested 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.
A260/A280 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 passing the column, add 210 µl of absolute alcohol to the

5. **Preheat 10 mM Tris-HCl (pH 9.0), ddH_2O or TE buffer at 70°C (500 µl/prep) for DNA elution at Step 10.**

6. **Add 210 µl of absolute ethanol or isopropanol to the sample from Step 4 and mix by vortexing.**

7. **Place a *GenCatch™* column onto a Collection Tube. Transfer <u>all</u> the mixture into the column. Centrifuge at 8000 rpm for 2 minutes. Place the column onto a <u>new</u> Collection Tube.**

8. **Wash the column <u>twice</u> with 0.5 ml WS Buffer by centrifugation at 8000 rpm for 2 minutes. Discard the flow-through after centrifugation.**

9. **Centrifuge the column at full speed for another 2 minutes to remove ethanol residue.**

10. **Place the column onto a new 1.5 ml tube and elute DNA with 200 µl of the preheated elution solution from Step 5.**

11. **Stand the column for 1-5 minutes, and centrifuge for 1-2 minutes to elute DNA.**

12. **Store eluted DNA at -20°C.**

II. Tissue Protocol:

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.**
   If the sample size is larger than 30 mg, Increase the amount of LYS Buffer proportionally.

2. **Add 20 µl Proteinase K to the sample. Mix immediately by vortexing for 20 seconds.**
   If RNA-free genomic DNA is desired,

add 10 µl of 50 mg/ml RNase A to the sample.

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 during incubation.**
   Ensure complete sample lysis, sample should appear translucent.

4. **Adjust the temperature to 70°C and incubate for another 20 minutes.**

5. **Preheat 10 mM Tris-HCl (pH 9.0), ddH_2O or TE buffer at 70°C (500 µl/prep) for DNA elution at Step 11.**

6. **Add 200 µl of EX Buffer to the sample, mix by vortexing and incubate at 70°C for 10 minutes.**
   If the sample remains undigested after incubation, centrifuge for 5 minutes at full speed and use only the supernatant in the following steps.

7. **Add 210 µl of absolute ethanol or isopropanol to the sample and mix by vortexing.**

8. **Place a *GenCatch™* column onto a Collection Tube. Transfer all the mixture into the column. Centrifuge at 8000 rpm for 2 minutes. Place the column onto a new Collection Tube.**

9. **Wash the column twice with 0.5 ml WS Buffer by centrifugation at 8000 rpm for 2 minutes. Discard the flow-through after centrifugation.**

10. **Centrifuge the column at full speed for another 2 minutes to remove ethanol residue.**

5 Troubleshooting Guide

The following guide addresses some of the most common problems.

Problem	Possible Reasons	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 of absolute alcohol to the sample.
	WS Buffer does not contain ethanol	Make sure that ethanol is added into the WS Buffer bottle when first open.
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	Before passing the column, add 210 µl of absolute alcohol to the sample.

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 sample.

3. Incubate at **30°C for 30 minutes**.

4.Pellet cells by centrifugation at 7500 rpm for 5 minutes. Resuspend the pellet in 200 µl LYS Buffer.

5. Follow the Tissue Protocol starting from Step 2.

VII. Virus Protocol:

1. To prepare viral DNA from blood or body fluid, the Blood Protocol is suggested.

2. To prepare integrated viral DNA, the Blood Protocol or Tissue Protocol is suggested.

11. Place the column onto a new 1.5 ml tube and elute DNA with 200 µl of 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 -20**°C**.

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III. Mouse Tail Protocol:

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 eppendorf tube.

Segment close to the tail tip is preferred. Segment away from the tip is thicker and takes longer time to lyse completely.

2. Add 20 µl Proteinase K and 200 µl LYS Buffer to the sample. Mix immediately by vortexing for 20 seconds.

If RNA-free genomic DNA is desired, add 10 µl of 50 mg/ml RNase A to the sample.

3. Incubate at **60°C for 1-4 hours** or overnight to lyse the tail tissue. Vortex or invert mix the sample every 20-30 minutes during incubation.

Ensure complete sample lysis, sample should appear translucent.

4. Proceed with the Tissue Protocol starting from Step 4.

IV. Paraffin-Embedded Tissue Protocol:

1. Cut a small section of paraffin-embedded tissue (about 25 mg) and put the sample into a 1.5 ml sterile 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.

4. Add 1 ml of 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°C** for 10 minutes.

6. Resuspend the pellet in 200 µl LYS Buffer.

7. Proceed with the Tissue Protocol starting from Step 2.

V. Bacteria Protocol:

A. Bacteria

1. Pellet log-phase grown bacteria of up to 10^9 (or up to 3 ml culture) at 7500 rpm 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 sample.

3. Add 20 µl Proteinase K and 200 µl EX Buffer to the sample. Mix immediately by vortexing for 20 seconds.

4. Incubate at **60°C for 30 minutes** to lyse the bacterial cells. Vortex or invert mix the sample every 5 minutes during incubation.

   Ensure complete sample lysis, sample should appear translucent.

5. Adjust the temperature to **70°C and incubate for another 30 minutes**.

6. Follow the Blood Protocol starting from Step 11.

B. Bacteria in biological fluids

1. Pellet cells by centrifugation at 7500 rpm for 10 minutes.
2. Resuspend the pellet in 200 µl LYS Buffer.
3. Follow the Tissue Protocol starting from Step 2.

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 centrifugation at 7500 rpm for 10 minutes.
3. Resuspend the pellet in 200 µl LYS Buffer.
4. Follow the Tissue Protocol starting from Step 2.

VI. Yeast Protocol:

1. Pellet log-phase grown yeast cells of up to 10^8 (or up to 3 ml culture) at 7500 rpm for 10 minutes.

2. Resuspend the pellet in 500 µl sorbitol