2260250-2/23

GenCatch™ Gel Extraction Kit

User's Guide for

Purification of DNA Frgaments From Agarose Gel

For Research Use Only

www.epochlifescience.com

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Quick Start Procedure

For Experienced Users Only.

First time users are strongly recommended to read through the detailed instruction protocol in section 4.

Before you start:

Add 24 ml (50 preps) or 120 ml (250 preps) 98-100% ethanol to WN and WS Buffer.

	Cut out Gel Slice
Solubalize in:	**3 Volumes GEX**
Heat to facilitate solubalization:	**Incubate at 50°C for 5-10'**
Bind to Column:	**Transfer to column**
Wash with:	**500 µl WN**
Wash with:	**500*µl WS**
Elute DNA in:	**15-30 µl EB**
	Down Stream Application

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Overview

GenCatch™ Advanced Gel Extraction Kit is designed to extract and purify DNA fragments of 70 bp up to 10 kb from normal and low-melting agarose gel. Low-melting agarose gel is not necessary for sample preparation. The kit is based on the long observed phenomenon that agarose gel dissolves in high concentration of chaotropic salts and DNA released can effectively bind to silica membrane. A single GenCatch™ Advanced Gel Extraction Column is capable of binding up to 20µg double stranded DNA with an average recovery of 70 to 98 % of 70 bp to 10 kb DNA fragments.

Preparation time: 10-15 minutes

Downstream Applications:

- Radioactive and Fluorescent sequencing
- Restriction enzyme digestion
- Labeling
- Ligation
- PCR
- Hybridization

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Product Contents

GenCatch™ Advanced Gel Extraction Kit contains sufficient reagents for 50 (Cat. No. 2260050) and 250 (Cat. No. 2260250) gel extraction applications respectively.

Catalog Number	2260050	2260250
GEX Buffer	50 ml	250 ml
WN Buffer	6 ml	30 ml
WS Buffer	6 ml	30 ml
Elution Buffer	5 ml	25 ml
GenCatch™ Column	50 pieces	250 pieces
Collection Tube	50 pieces	250 pieces

Add 24 ml (50 preps) or 120 ml (250 preps) 98-100% ethanol to WN and WS buffer bottle when first opened.

Storage Conditions:
Store at room temperature

All components are guaranteed for 24 months from the date of purchase, when stored under specified conditions and used as described in this manual. Long term storage of Buffer GEX may harden the HDPE plastic bottle. However this will not adversely affect the performance of the kit. ***GenCatch™*** *Advanced* Gel Extraction columns should be kept sealed in the zip lock bag provided during storage and away from any heating source.

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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: ***support.epochbiolabs.com***

Before you start:

Add 24 ml (50 preps) or 120 ml (250 preps) 98-100% ethanol to Buffer WN and WS before use (refer to instructions on bottle label).

Notes: For buffer exchange or DNA clean up directly from solutions (ligation, restriction digestion, random labeling, vector dephosphorylation etc.), first dilute the reaction to 200 µl using TE buffer and then add 3 volumes of Buffer GEX, mix and proceed directly to step 4 of this protocol.

I. Using a Centrifuge:

1. Use a clean, sharp scalpel or razor blade to excise the gel slice containing the DNA fragment of interest under the illumination of long wave length UV light.
   Minimize the size of the gel slice by removing extra agarose. Expose to short wavelength UV light may damage DNA and introduce mutation.

2. Measure the weight of gel slice (about 100-350 mg) and place it into a sterile 1.5-ml or 2-ml centrifuge tube. Add 3 volumes GEX Buffer to 1 volume of gel (taken 300 mg of gel slice as roughly equal to 300 µl).
   Cutting the gel slice into small pieces can facilitate dissolution. For higher percentage agarose gel (→2 %), add GEX Buffer as 4 to 6 volumes of the gel slice (100 mg = 0.1 ml).

3. Incubate at 55°C for 5 to 10 minutes until the gel is completely dissolved. Invert the tube every 1-2 minutes during incubation.
   Ensure that the gel has been completely dissolved before proceeding to step 4.

4. Place a *GenCatch™* Advanced Gel Extraction Column onto a collection tube. Load no more than 0.7 ml dissolved gel mixture onto the column. Centrifuge for 30 seconds at 5000 rpm. Discard the flow-through.

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5. Repeat step 4 for the rest of the mixture if total volume of the dissolved gel slice is more than 0.7 ml.

6. Wash the column once with 0.5 ml of WN Buffer by centrifuging for 60 seconds at full speed. Discard the flow-through.

7. Wash the column once with 0.5 ml of WS Buffer by centrifuging for 60 seconds at full speed. Discard the flow-through.
   Ensure that ethanol has been added into WS Buffer bottle when first open.

8. Centrifuge the column at 12000 rpm for another 1 minute to remove residual ethanol.
   Residual ethanol can affect the quality of DNA and inhibit subsequent enzymatic reactions.

9. Place the column onto a new 1.5-ml centrifuge tube. Add 15-30 μl of Elution Buffer 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.

10. Stand the column for 2 minutes (optional, for better elution) and centrifuge 60 seconds at full speed to elute DNA. Store DNA at –20 °C.

II. Using a Vacuum Manifold:

The following protocol uses a vacuum manifold (not provided in this kit).

Follow Step 1-3 in protocol I and proceed to step 4.

4. Insert a *GenCatch™* Advanced Gel Extraction 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 onto the column.

5. Apply vacuum to draw all the liquid into the manifold. Load the rest of the mixture.

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6. Wash the column once with 0.5ml of WN Buffer by re-applying vacuum to draw all the liquid.

7. Wash the column once with 0.5ml of WS Buffer by re-applying vacuum to draw all the liquid.

Place the column onto a Collection Tube and proceeds to step 8 in protocol I all the way to the end.

* Vac-man is a trademark of Promega Inc.

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5 Troubleshooting Guide

The following guide addresses some of the most common problems. A database of user raised questions and answers are being build at support.epochbiolabs.coom.

Problem	Possible Reasons	Solution
Gel slice hard to dissolve	Use high percentage agarose gel	Add GEX Buffer as 6 volumes of the gel slice → 2.5%. Incubate with mixing every 1-2 minutes until complete.
	Gel slice is too big (more than 200 mg)	Use more than one column for gel slice more than 200 mg.
Low recovery of DNA fragment	Ineffective DNA elution	DNA elution does not take place well at acidic conditions. Make sure that ddH2O 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 30 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.

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	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.
Lower recovery of DNA fragment	Size of DNA fragment is more than 5 kb	Use elution solution preheated to 60°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°C for 2 minutes and let it cool slowly to room temperature. Reannealed 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.

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