2360250-2/23

GenCatch™ PCR Cleanup Kit

User's Guide for

PCR Reaction Purification

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.

	PCR Reaction
Solubilize in:	**500 µl PX**
Bind to Column:	**Load and Spin in 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 PCR Cleanup Kit is designed to extract and purify DNA fragments of 100 bp up to 10 kb from PCR or other enzymatic reactions. Specific binding and subsequent efficient elution of DNA from silica membrane can be achieved by simple centrifugation steps without phenol/chloroform. A single GenCatch™ Column is capable of binding up to 10 µg DNA with recovery efficiency up to 95 % for 100 bp to 10 kb DNA fragments.

Preparation time: 5-10 minutes

Downstream Applications:

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

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Product Contents

GenCatch™ Advanced PCR Cleanup Kit contains sufficient reagents for 50 (Cat. No. 2360050) and 250 (Cat. No. 2360250) PCR cleanup applications respectively.

Catalog Number	2360050	2360250
PX Buffer	30 ml	150 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 PX may harden the HDPE plastic bottle. However this will not adversely affect the performance of the kit. *GenCatch™ Advanced* PCR Cleanup 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).

I. Using a Centrifuge:

1. Pipet 10 -100 µl PCR product (make sure that mineral oil is not taken if PCR machine without heated cover is used) or DNA solution after enzymatic reaction to a new 1.5 ml centrifuge tube. Add 0.5 ml PX Buffer and mix well.

2. Place a *GenCatch™* Column onto a Collection Tube. Add all the mixture from step 1 into the column.
   Load no more than 0.7 ml mixture into the column each time.

3. Centrifuge at 5000 RPM for 30-60 seconds. Discard the flow-through.

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

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

6. Centrifuge the column at full speed for another 1 minutes to remove ethanol residue.
   It is important to remove ethanol residue, residual ethanol may inhibit subsequent enzymatic reactions.

7. 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

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solution is dispensed onto the center of the membrane and is completely absorbed.

8. Stand the column for 2 minutes and centrifuge for 1 minute to elute DNA.

9. Store DNA at–20 ℃.

II. Using a Vacuum Manifold:

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

1. Pipet 10 -100 µl PCR product (make sure that mineral oil is not taken if PCR machine without heated cover is used) or DNA solution after enzymatic reaction to a new 1.5 ml centrifuge tube. Add 0.5 ml PX Buffer and mix well.

2. Insert a *GenCatch™* Column into the luer-lock of a vacuum manifold (e.g. Promega's Vac-man*). Add all the mixture from step 1 into the column.
   Load no more than 0.7 ml of the mixture onto the column.

3. Apply vacuum to draw all the liquid into the manifold.

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

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

6. Place the column onto a Collection Tube. Centrifuge the column at full speed for 1 minutes to remove residual ethanol and proceeds to step 7 all the way to the end in protocol I.

* 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
Low recovery of DNA fragment	DNA solution used is more than 100 µl	Divide loading the sample into two or more columns. If DNA to be cleaned up is diluted, more than 100 µl solution can be used per column. Add 5 µl of more PX Buffer for each 1 µl of extra DNA solution (e.g. add 600 µl PX Buffer to 120 µl DNA solution).
	DNA solution used is of pH less than 7.5	Add 10µl 0.1 M Tris-HCl (pH 9.0) into the DNA solution before adding PX Buffer.
	Overload the column with too much DNA	Higher recovery is attained from lower amount of loaded DNA. Split loading of high amount of DNA into two or more columns.
	Ineffective DNA elution	DNA elution does not take place well at acidic conditions. Make sure that water or buffer 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.
	Size of DNA product is more than 5 kb	Use elution solution preheated to 60°C.

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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 1 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.

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