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Introduction

This fast and reliable kit is designed to recover DNA from agarose gels, and purify DNA fragments from PCR, RFLP, phosphorylation, labeling, ligation, hybridization and other enzymatic reactions. DNA fragments from 100 bp to 20 kb can be purified using the ezBind™ mini column with over 80-90 % recovery.

Storage and Stability

All components can be stored at room temperature. All kit components are guaranteed for 12 months from the date of purchasing.

Kit Contents

Catalog#	**DC3511-00** **DC3514-00**	**DC3511-01** **DC3514-01**	**DC3511-02** **DC3514-02**	**DC3511-03** **DC3514-03**
Preps	4	50	250	100
Buffer GC	3 mL	25 mL	120 mL	50 mL
DNA Wash Buffer*	2 mL	15 mL	3 x 24 mL	2 x 15 mL
Elution Buffer	1 mL	10 mL	20 mL	15 mL
ezBind Mini Columns	4	50	250	100
1.5mL Collection Tubes	4	50	250	100
User Manual	1	1	1	1

Safety Information

Buffer GC contains acidic acid and chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste.

Before Starting

Prepare all components and get all necessary materials ready by examining this instruction booklet and become familiar with each steps.

Important

- ☑ Add absolute ethanol to DNA Wash Buffer as Follows:
  DC3511/DC3514-00: Add 8 mL 100% ethanol before use.
  DC3511/DC3514-01: Add 60 mL 100% ethanol before use.
  DC3511/DC3514-02: Add 96 mL 100% each to ethanol before use.
  DC3511/DC3514-03: Add 60 mL 100% each to ethanol before use.
- ☑ A gel slice of 100 mg equals to a volume of 100 μL.
- ☑ Buffer GC may form precipitates under cool ambient condition. Warm up the buffer at 37 ℃ to dissolve before use.
- ☑ Pre-warm aliquots of Elution Bufer or ddH_2O at 55-60 ℃ waterbath.

Materials supplied by users

- ☑ Tabletop microcentrifuge and 1.5 mL microtubes.
- ☑ 55-60 ℃ waterbath.
- ☑ Vacuum manifold if use vacuum protocol.
- ☑ 96~100 % ethanol.
- ☑ Isopropanol for DNA fragment less than 200 bp.

Perform all steps including centrifugation at room temperature!

PCR/Gel Extraction Spin Protocol

Fresh TAE buffer as running buffer is recommended. Reusing running buffer will result the increase of the pH and then reduce yields

1. ***For agarose gel*:** Excise the DNA fragment from the agarose gel and weigh it in a 1.5 mL microtube. Add **1 volume** of **Buffer GC** to **1 volume** of gel to the 1.5 mL microtube and incubate the mixture at 55-60℃ for 8-10 min. Mix the tube by tapping the bottom every 2 min till the gel has melted completely. Cool the tube to room temperature.

   For cycle-pure (PCR reaction): Add ***2 volumes*** of ***Buffer GC*** to ***1 volume*** of the PCR reaction and mix completely by vortexing. Briefly spin the tube to collect any drops from the inside wall and tube lid. For PCR products less than 200 bp, add 5 volumes of Buffer GC to 1 volume of PCR reaction

   Note: A gel slice of 100 mg approximately equals to 100 μL.
   Note: For DNA fragment less than 200 bp, add **1 volume** of **isopropanol**.

2. Transfer up to **700 μL DNA/Buffer GC** mixture to a spin column with a collection tube. Centrifuge at 13,000 x g for 1 min at room temperature. Discard the flow-through and put the column back to the collection tube. Repeat this step to process the remaining solution.

3. Add **600 μL DNA Wash Buffer** to the column and centrifuge at 13,000 x g for 30 s at room temperature. Discard the flow through and insert the column, with the lid open, back to the collection tube. Repeat step "3".

   Note: Ensure that ethanol has been added to DNA Wash Buffer as instructed .

4. Centrifuge the empty DNA column, **with the lid open,** at 13,000 x g for 2 min to dry the ethanol residue in the matrix.

   Note: The residual ethanol will be removed more efficiently with the column lid open during centrifugation. Otherwise the DNA column should be set at room temperature for a few miniutes until there is no alcoholic.

5. Place the column into a clean 1.5 mL micocentrifuge tube and add **30-50 μL** pre-warmed (60°C) **Elution Buffer or ddH_2O** to the center of the column. Incubate at room temperature for 1 min. Centrifuge at 13,000 x g for 1 min to elute the DNA. Reload the eluted DNA solution to the column for a second

elution.

Note: Pre-warm elution buffer or ddH$_2$0 at 60 ℃ and incubate the column at 60 ℃ for 5 min after adding Elution Buffer or ddH20 will increase the DNA yield.

Note: For fragment larger than 8 kb, incubate the column at 60 ℃ for 15 min after adding Elution Buffer or ddH$_2$0 will increase the DNA yield.

Note: The first elution normally yields 60-70% of the DNA bound. Reload the eluted DNA solution to the column for a second elution will yield another 20% of the DNA that makes the total yield up to 90%.

PCR/Gel Extraction Vacuum/Spin Protocol

1. Follow the instruction described on step 1 on page 4. Briefly spin the tube to collect any drops from the inside wall and tube lid.

2. Prepare the vacuum manifold according to manufacturer's instructions. Attach the spin column to the manifold**.**

3. Load the **Gel or PCR reaction/ Buffer GC solution** to a spin column that is attached to the manifold**.** Turn on the vacuum to let the solution pass through the column.

4. Wash the column by adding **600 µL DNA Wash Buffer.** Repeat **step 5.** Vacuum the column for 1 min.

5. Turn on the vacuum, dry the empty column for 5 mins.

6. Put the column to a clean 1.5 mL tube and add **30-50 µL Elution Buffer or ddH$_2$O** to the column. Incubate at room temperature for 2 min. Centrifuge the tube at 13,000 x g for 1 min to elute DNA.

Note: Pre-warm Elution Buffer or ddH$_2$0 at 60 ℃ and incubate the column at 60 ℃ for 5 min after adding elution buffer or ddH$_2$0 will increase the DNA yield.

Note: The first elution normally yields 60-70% of the DNA bound. Reload the eluted DNA solution to the column for a second elution will yield another 20% of the DNA that makes the total yield up to 90%.

琼脂糖凝胶/PCR 产物纯化简明步骤
（DC3511/DC3514）

(详细内容请参考说明书英文部分)

实验前准备：

- 第一次使用本产品，请按照瓶子上的标签或者以下标准加入无水乙醇到 DNA Wash Buffer 中。

  DC3511/DC3514-00: 加入8 mL 无水乙醇到DNA Wash Buffer中。

  DC3511/DC3514-01: 加入60 mL 无水乙醇到DNA Wash Buffer中。

  DC3511/DC3514-02: 每瓶DNA Wash Buffer中加入96 mL 无水乙醇。

  DC3511/DC3514-03: 每瓶DNA Wash Buffer中加入60 mL 无水乙醇。

- 100 mg 的凝胶只需要 100 µL Buffer GC。
- Buffer GC 在低温运输或者保存过程中可能会生成沉淀，如果有沉淀生成，请在 37 ºC 溶解再使用。
- 洗脱前，在 55-60 ºC 水浴预热 Elution Buffer。

操作步骤

请使用新鲜配制的TAE或者TBE缓冲液，使用多次的缓冲液pH会偏高从而影响DNA回收效率或带来污染。

1. **琼脂糖凝胶产物纯化：**从凝胶上割下带有目的片段的凝胶块到一个1.5mL或者2.0mL离心管中，加入**1倍体积**的**Buffer GC** (称量或者估算凝胶的重量，确保加入不少于1倍体积的Buffer GC），置于55-60 ºC水浴中8-10分钟左右，期间颠倒混匀几次，直至凝胶块完全溶化。冷却离心管至室温。

   PCR产物纯化：加入**2倍体积的 Buffer GC**(如100的PCR反应液，加入200µLBuffer GC)，混合混匀。瞬时离心，将溶液收集到底部。纯化小于200 bp的片段，加入5倍体积的Buffer GC到1倍体积的PCR反应液。

   注意：100mg凝胶块需加入100µL的Buffer GC，确保加入不少于1倍体积的Buffer GC。

   注意：纯化小于200 bp的片段，加入1倍体积的异丙醇（100mg凝胶块或100µLPCR反应液，加入100µL的异丙醇）。

2. 转移以上混合液（每次不超过700 µL ）至一个带有收集管的吸附柱中，室温下，13,000 x g下离心1分种，弃废液，将离心柱放回收集管中。重复步骤“2”，直至剩余的混合液全部通过吸附柱。

3. 加入**600 µL DNA Wash Buffer** 至吸附柱中，室温下在13,000 x g下离心30秒，倒掉收集管中的废液，将吸附柱放回到收集管中。重复步骤3。
   注意：DNA Wash Buffer作为浓缩液提供，使用前确保加入无水乙醇。

4. 室温下，13,000 x g，将吸附柱开盖离心2分钟，去除残留的乙醇。
   注意：开盖离心更有利于乙醇的去除，乙醇的残留将影响下游实验，可以适当延长离心时间，并且离心后开盖空气中放置几分钟。

5. 转移吸附柱至1.5mL收集管中，加入**30-50 µL** 60ºC 预热的**Elution Buffer（10mM Tris-HCl, pH8.5）或ddH_2O到吸附柱膜中央，**室温放置1分钟。13,000 x g 离心1分种以洗脱DNA。如果想提高收获量，将洗脱液加回到吸附柱中重新洗脱一次。

   注意： 将洗脱液置于60 ºC预热，加入洗脱液后吸附柱置于60ºC放置5分钟后再进行洗脱，将会显著提高DNA回收率。
   注意： 对大于8kb的片段，加入洗脱液后将吸附柱置于60ºC放置15分钟后再进行洗脱，将会显著提高DNA回收率。
   注意：第一次洗脱会收集到约60-70%的DNA，将洗脱液加回到柱子中进行二次洗脱，将会收集到另外20%的DNA，DNA回收率可以达到90%以上。DNA回收率会片段大小不同而有差异。

Trouble Shooting Guide

Problems	**Possible reasons**	**Suggested improvements**
Low DNA yield	1. Not enough Buffer GC	1. Determine the volume of Buffer GC to be used correctly as instructed.
	2. Agarose gel doesn't melt completely	2. Make sure to set the water bath to 55-60°C to allow gel to melt completely. Add more Buffer GC if necessary.
	3. Reused electrophoresis buffer with increased pH.	3. Use fresh electrophoresis buffer.
	4. Fragment < 200 bp	4. Add isopropanol as instructed.
	5. Fragment >10 kb	5. Incubate the column (after adding ddH_20 or Elution Buffer) at 60°C for 15 min before elution.
No DNA yield	Forgot to add ethanol to DNA Wash Buffer	Add absolute ethanol to DNA Wash Buffer as instructed before use.
DNA sample floats out of well while loading agarose gel	Ethanol was not completely removed from the column following wash step	After the wash step, centrifuge the empty column with the lid open at top speed for 1-3 min. Repeat once.
Column clogged	Agarose gel doesn't melt completely	Make sure to melt the gel at 55-60°C before loading the sample to DNA column.

*** FOR RESEARCH USE ONLY.**