Ver: 2503

DNA Gel/PCR Purification Midiprep Kit

（BW-DC3521）

Contents

Kit Contents

Catalog#	BW-DC3521-00	BW-DC3521-01	BW-DC3521-02
Preps	4	10	25
Buffer GBL	5 mL	12 mL	30 mL
Buffer GC	10 mL	50 mL	120 mL
Buffer KB	15 mL	35 mL	80 mL
DNA Wash Buffer*	12 mL	36 mL	2 x 48 mL
Elution Buffer	2 mL	5 mL	10 mL
Buffer C1	1 mL	1 mL	1 mL
Midi Columns	4	10	25
User Manual	1	1	1

* Add 28 mL (DC3521-00) or 84 mL(DC3521-01) or 112 mL (DC3521-02) 100% ethanol to DNA Wash Buffer before use.

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 50-90 % recovery.

Storage and Stability

All components can be stored at room temperature (15-25℃). All kit components are guaranteed for 12 months from the date of production.

Before Starting

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

Important Notes

- **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°C to dissolve before use.
- Pre-warm aliquots of Elution Buffer or ddH_2O at 55-60°C water bath.

Materials supplied by users

- Tabletop microcentrifuge and 15 mL microtubes.
- 55-60°C water bath.
- Vacuum manifold if use vacuum protocol.
- 96~100 % ethanol.
- Isopropanol for DNA fragment less than 200 bp or more than 4kb.

Perform all steps including centrifugation at room temperature!

Important Notes

- **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°C to dissolve before use.
- Pre-warm aliquots of Elution Buffer or ddH_2O at 55-60°C water bath.
- Buffer GC contains an integrated pH indicator, allowing easy determination of the optimal pH for DNA binding. DNA adsorption requires pH ≤7.5, and the pH indicator in the buffers will appear yellow in this range. If the pH is >7.5, and the pH indicator in the buffers will appear pink in this range. This means that the pH of the sample exceeds the buffering capacity of Buffer GC, and DNA adsorption will be inefficient. In these cases, the pH of the binding mixture can easily be corrected by adding **10µL Buffer C1** to per sample, before proceeding with the protocol.

Materials not Supplied

- Tabletop microcentrifuge and 15 mL microtubes.
- 55-60°C water bath.
- 96~100 % ethanol.
- Isopropanol for DNA fragment less than 200 bp or more than 4kb.

Perform all steps including centrifugation at room temperature!

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 waste.

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. Column equilibration: Place a **Midi Column** in a clean collection tube, and add **1mL Buffer GBL** to column. Centrifuge for 1 min at 12,000 rpm in a table-top centrifuge. Discard the flow-through, and set the column back into the collection tube. (Please use freshly treated spin column).

2. ***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

   ***For agarose gel*:** Excise the DNA fragment from the agarose gel and weigh it in a 15 mL microtube. Add **1 volume** of **Buffer GC** to **1 volume** of gel to the 15 mL microtube and incubate the mixture at 60°C for10-20 min. Mix the tube by tapping the bottom every 2 min till the gel has melted completely. Cool the tube to room temperature.

   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**.
   Note: For >2% gel, add 2 volume GC.
   Note: The maximum amount of gel slice per Midi column is 4g; for gel slices >4g, use more than one Midi column.
   Note: For DNA fragment less than 200 bp/ more than 4000bp, add 1 volume of isopropanol.

3. Load the (≤4mL) **DNA/Buffer GC** mixture to the pretreated spin column with a collection tube. Centrifuge at 12,000 rpm 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.

4. **Optional: For agarose gel:** add **3 mL Buffer KB** to the column and centrifuge at 12,000 rpm for 30 s. Discard the flow through and insert the column.

5. Add **4 mL DNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 1 min at room temperature. Discard the flow through and insert the column, with the lid open, back to the collection tube. Repeat step “5”.

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

6. Centrifuge the empty DNA column, **with the lid open,** at 12,000 rpm 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.

7. Place the column into a clean 15 mL micocentrifuge tube and add **200 µL** pre-warmed (60°C) **Elution Buffer or ddH$_2$O** to the center of the column. **Incubate at room temperature for 1 min.** Centrifuge at 12,000 rpm 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°C and incubate the column at 60°C 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 °C 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%.

胶回收/PCR 产物纯化简明步骤（DC3521）

(详细内容请参考说明书英文部分)

使用前请注意：

- 使用前请分别在 DNA Wash Buffer 中加入 8 mL (DC3521-00) 或者 12mL(DC3521-01) 或者 60 mL (DC3521-02) 100% 乙醇。
- 溶解 100 mg 的琼脂糖凝胶，约需要 100 µL Buffer GC。
- 溶胶液 GC 在较低温度下易沉淀，若温度较低，出现沉淀，使用前请在 37 °C 加热几分钟，至沉淀溶解后再使用。
- 本试剂盒所有操作步骤均在室温下进行。
- Buffer GC 中含有 pH 指示剂，若样本与 Buffer GC 混合后溶液呈紫色，向体系中加入 **10µL Buffer C1**，以确保溶液 pH 低于 7.5。

用户自备材料:

- ☼ 100% 乙醇
- ☼ 台面离心机和15 mL 微型离心管
- ☼ 55-60 °C 水浴(胶回收时用)
- ☼ 小于200 bp或大于4kb的片段的可加异丙醇。

PCR产物纯化/胶回收离心法操作步骤：

1. 柱平衡：向吸附柱**Midi Columns** 中加入**1mL Buffer GBL**，12,000 rpm 离心1分钟，弃去收集管中的滤液，将吸附柱重新放回收集管中备用。（处理完请于当天使用）。

2. **PCR产物纯化**：在**1倍体积的**PCR反应物中加入**2倍体积的 Buffer GC**，涡旋充分混匀。对小于200 bp的PCR产物，加入5倍体积的Buffer GC。

胶回收：将含DNA片段的琼脂糖凝胶切下，转至15 mL离心管中，再加入**1倍体积**的**Buffer GC** (100 mg的凝胶加入100 µL Buffer GC)。在60 ºC下培育10-20分钟左右，且每隔2分种轻弹试管底部，直至胶溶解完全，放置使其冷却至室温。

建议:

- 为取得最好的实验结果，请使用新鲜配制的电泳缓冲液制胶和电泳。
- 切胶时，尽量缩短在紫外灯下照射的时间，以减少紫外对DNA的损伤，并尽量切除不含DNA的凝胶。
- 若DNA片段小于200 bp或大于4kb，请加入1体积的异丙醇。
- 对于大于 2%的凝胶，加入 2 体积的 GC-A Buffer；
- 每个 Midi Column 最大承载 4g 凝胶，对于大于 4g 凝胶的样本需增加一个 Midi Column 柱子；

3. 一次转移不超过**4 mL DNA/Buffer GC** 混合溶液至预处理的离心柱中，室温下，12,000 rpm下离心1分种，弃废液，将离心柱放回收集管中。重复此步使剩余的溶液通过柱子。

4. 加入**3 mL Buffer KB**，室温下12,000 rpm离心1 min，弃废液。

5. 加入**4 mL DNA Wash Buffer** （确保已将乙醇按说明书要求加入DNA Wash Buffer中），室温下在12,000 rpm离心1分钟，弃废液。重复步骤5。

6. 12,000 rpm开盖再次离心2分钟，以去除柱中残余的乙醇。
   注意：此步操作对DNA的产率多少极为关键。

7. 将柱子放入干净的1.5 mL试管中，向柱中加入在60ºC 下预热的**200 µL Elution Buffer或ddH$_2$O**。在室温下放置1分钟。12,000 rpm 离心1分种以洗脱DNA。将洗脱液重新上柱，再次离心洗脱。

   建议：将洗脱缓冲液预热到65 ºC，加洗脱液后将柱子整个温育5分钟后再离心洗脱可以增加DNA回收效率。
   对大于8kb的片段，加洗脱液后将柱子整个温育15-30分钟可以提高回收效率。

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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.

Limited Use and Warranty

This product is intended for *in vitro* research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product.

For technical support or learn more product information, please contact us or visit our website.

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