PCR / Gel Extraction Kit

User Guide

FOR RESEARCH USE ONLY

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1. Introduction

Quintech PCR / Gel Extraction 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.

Our proprietary DNA binding system results in a highly efficient and reversible binding of DNA to the ezBind mini column, with proteins and other impurities removed by wash buffer. Nucleic acids are then eluted with sterile water or elution buffer.

2. Kit Components & Safety Information

Catalog#	QT100-1201S SAMPLE	QT100-1202 50 Preps	QT100-1201 250 Preps
Preps	6	50	250
ezBind Mini Columns	6	50	250
Buffer BK	3.5 ml	30 ml	150 ml
Buffer G1[+]	6.0 ml	25 ml	125 ml
Buffer WA*	3.5 ml with ethanol added	12 ml	3 x 24 ml
Buffer EB	500 µL	10 ml	30 ml
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*48 ml (50 preps) or 96 ml (250 preps) 95-100 % ethanol to Buffer WA before use.

[+]Buffer G1 contains acidic acid and chaotropic salts, which may form reactive compounds when in contact with bleach. Do not add bleach or acidic solutions directly to the preparation waste. Please handle with gloves and protective eyewear.

3. Storage

Quintech PCR / Gel Extraction Kit should be stored dry at room temperature. Product performance and quality is maintained for kits stored up to 12 months at room temperature. For longer storage, kits can be stored at 2-8°C. Should any precipitate be observed in the buffers, redissolve at 37°C.

4. Supplies Needed

Materials to be supplied by user:
1.5 ml microcentrifuge tubes
95-100% ethanol
Isopropanol for DNA fragment less than 200bp
High speed microcentrifuge (13,000 rpm)
55-60°C waterbath
Vacuum manifold

Materials to be prepared:
48 ml (50 preps) or 96 ml (250 preps) 95-100% ethanol to Buffer WA before use

A gel slice of 100 mg approximately equals to a volume of 100 µl

For DNA fragments less than 200 bp, add **1 volume of isopropanol**

Buffer G1 may form precipitate below room temperature. It is critical to warm up the buffer at 37℃ to dissolve the precipitate before use

Pre-warm aliquots of Buffer EB of ddH_2O in the 55-60°C waterbath

Perform all steps including centrifugations at room temperature

5. PCR / Gel Extraction: Spin Protocol

Fresh TAE buffer is recommended as running buffer. Reusing running buffer will result in the increase of pH and ultimately reducing yield.

1. Add **500 µl Buffer BK** into the spin column, let it stand for 6 min. Spin the column with BK at 13,000 rpm for 2 min.

2. *For **cycle-pure (PCR** reaction)*
Add **2 volumes of Buffer G1** to **1 volume of 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 G1** to **1 volume of PCR reaction.**

   For agarose gel
   Excise the DNA fragment from the agarose gel and weigh it in a 1.5 ml tube. Add **1 volume of Buffer G1** to **1 volume of gel** to the 1.5 ml tube and incubate the mixture at 55-60°C for 8 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
   - For DNA fragments less than 200 bp, add **1 volume of isopropanol**

3. Transfer up to 700 µl DNA / Buffer G1 mixture to a spin column with a collection tube. Incubate mixture in column for at least 2 min before spinning at 13,000 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. Add **500 µl Buffer WA** to the column and centrifuge at 13,000 rpm for 30 min at room temperature. Discard the flow-through and insert the column, with the lid open, back to the collection tube. Repeat step 4.

   Note: Ensure that ethanol has been added to Buffer WA as instructed

5. Centrifuge the empty DNA column, **with the lid open**, at 13,000 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

6. Place the column into a clean 1.5 ml tube and add **30-50 µl** pre-warmed (60°C) **ddH20** or **Buffer EB** to the center of the column. **Incubate at room temperature for 1 min**. Centrifuge at 13,000 rpm for 1 min to elute the DNA. Reload the eluent into the column for a second elution and spin at same speed for 2 min.

Note:

- Pre-warm Buffer EB or ddH_2O at 60°C and incubate the column at 60°C for 5 min will increase the DNA yield
- The first elution typically yields 60-70% of the bounded DN. The second elution will yield an additional 20% of the DNA, bringing the yield to 90%

6. PCR / Gel Extraction: Vacuum Protocol

1. Add **500 µl Buffer BK** into the spin column, let it stand for 2 min, centrifuge for 2 min at 13,000 rpm.

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

3. Load the **Gel** or **PCR reaction / Buffer G1 solution** to a spin column attached to the manifold. Turn on the vacuum to let the lysate pass through the column.

4. Wash the column by adding **500 µl Buffer WA** into the spin column and allow the lysate to pass through the column by vacuum for 1 min.

5. Transfer the column, **with the lid open**, to a 1.5 ml collection tube and centrifuge at 13,000 rpm for 2 min.

Note: The residual ethanol will be removed more efficiently with the column lid open during centrifugation

6. Place the column into a clean 1.5 ml tube and add **30-50 µl** pre-warmed (60°C) **ddH20** or **Buffer EB** to the center of the column. **Incubate at room temperature for 1 min**. Centrifuge at 13,000 rpm for 1 min to elute the DNA. Reload the eluent into the column for a second elution.

Note:

- Pre-warm Buffer EB or ddH_2O at 60°C and incubate the column at 60°C for 5 min will increase the DNA yield
- The first elution typically yields 60-70% of the bounded DN. The second elution will yield an additional 20% of the DNA, bringing the yield to 90%

7. Troubleshooting Guide

Problem	Possible	Recommended Solution
Low DNA yield	Insufficient Buffer G1	Determine the volume of Buffer G1 to be used as instructed
	Agarose gel does not melt completely	Ensure that the water bath is set at 55-60°C to allow gel to melt completely. Add more Buffer G1 if necessary
	Reused electrophores is buffer with increased pH	Use fresh electrophoresis buffer
	Fragment <200 bp	Add isopropanol as instructed
	Fragment >10 bp	Incubate column (after adding ddH_2O or Buffer EB) at 60°C for 15 min before elution
No DNA yield	Ethanol not added to Buffer WA	Add absolute ethanol to Buffer WA as instructed before use
Clogged column	Agarose gel does not melt completely	Ensure complete melt of gel at 55-60°C before loading sample to column
DNA sample floats out of well while loading into agarose gel	Ethanol traces were not completely removed from column during wash step	After wash step, centrifuge the empty column with the lid open at 13,000 rpm for 1 to 3 min. Repeat (if necessary)

8. Ordering Information

Catalog #	Description
QT100-1201	Quintech PCR / Gel Extraction Kit, 250 preps Includes 250 ezBind mini columns, reagents and user guide
QT100-1202	Quintech PCR / Gel Extraction Kit, 50 preps Includes 50 ezBind mini columns, reagents and user guide
Kit Components	
QT100-8101C	ezBind Mini Columns, 250 preps
QT100-8102C	ezBind Mini Columns, 50 preps
QT100-8201BK	Buffer BK, 150 ml, 250 preps
QT100-8202BK	Buffer BK, 30 ml, 50 preps
QT100-8215G1	Buffer G1, 125 ml, 250 preps
QT100-8216G1	Buffer G1, 25 ml, 50 preps
QT100-8217WA	Buffer WA, 24 ml, 3, 250 preps
QT100-8218WA	Buffer WA, 12 ml, 50 preps
QT100-8213EB	Buffer EB, 30 ml, 250 preps
QT100-8214EB	Buffer EB, 10 ml, 50 preps

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Simplifying Sample Purification

www.quintechlifesciences.com

Quintech Life Sciences Pte Ltd
No. 9 Chin Bee Avenue Singapore 619933
Tel: +65 6376 2093 Fax: +65 6376 2015
Email: sales@quintechlifesciences.com

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