Cat.NO. 101081

Store the kit at 15 to 25℃

Genorise Gel DNA Purification Mini Kit
PCR Clean Kit 200

For Research Use only

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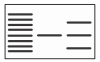
PCR clean-up, Gel extraction

Protocol-at-a-glance

	PCR clean-up	Gel extraction
1 PCR clean-up: Adjust binding condition Gel extraction: Excise DNA fragment / Solubilize gel slice	 100 µL PCR 300µLBuffer GGA 150µL Buffer GGB （Or 300 µL Buffer GP）	 100 mg gel 300 µL Buffer GGA 55℃ 5-10 min 150µL Buffer GGB
2 【*Optional steps*】 Prepare The Column	 	250 µL Buffer GL 10,000 x *g* 1 min
3 Bind DNA	 	10,000 x *g* 1 min
4 Wash silica membrane 5 Wash silica membrane	 	500 µL Buffer GW2 700 µL Buffer GW2 10,000 x *g* 1 min
6 Dry silica membrane		10,000 x *g* 1 min
7 Elute DNA	 	10–25 µL Buffer GE RT 1 min 10,000 x *g* 1 min

2.2 Protocol for PCR clean-up

① **For DNA clean-up from enzymatic reactions using this protocol,**

two methods to choose from one of the following

@USE **Buffer GGA** and **Buffer GGB**

●add 3 volumes of **Buffer GGA**， Add **Buffer GGB**0.5 volume of **GGA**

For example, if the PCR product is 100µL， add 300µL **Buffer GGA**,Add 150µL **Buffer GGB**

●mix, proceed with **step2.1** ②（**page 6**）of the protocol.

@USE **Buffer GP**

●add 3 volumes of **Buffer GP**

For example, if the PCR product is 100µL， add 300µL **Buffer GP**.

●mix, proceed with **step2.1** ②（**page 6**）of the protocol.

3.Troubleshooting

Problem	Possible cause and suggestions
Incomplete Lysis of Agarose Slices	*Time and temperature* •Check incubation temperature. Depending on the weight of the gel slice, incubation (step ①) can be prolonged up to 20 min. Vortex every 2 min and check integrity of the gel slice. Very large gel slices can be crushed before addition of **Buffer GGA**.
Low DNA yield	*Reagents not prepared properly* •Add indicated volume of 95–100% ethanol to Buffer GW2 Concentrate and mix well before use.
	Incompletely dissolved gel slice •Increase time or add another two volumes of **Buffer GGA** and vortex the tube every 2 minutes during incubation at 50°C. Small pieces of gel are hardly visible and contain DNA that will be lost for purification.
	Insufficient drying of the silica membrane •Centrifuge 5 min at 10,000 x g or incubate column for 2–5 min at 70°C before elution to remove ethanolic Buffer GW2 completely. Ethanolic contaminations are also indicated by gel-loading problems (samples float out of gel slots). remove the spin cup carefully from centrifuge and collection tube and avoid contact of spin cup with flow-through.
	Not enough elution buffer •Especially when larger amounts of DNA(>5µg) are bound, increase elution buffer volume up to 50 µL.
	Isolation of large DNA fragments •Preheat Buffer GE to 70°C, and incubate on the silica membrane at room temperature for 2 min before centrifugation.

② 【*Optional steps*】 **Prepare the DNA Binding Column:**

- Insert one DNA Binding Column into one Collection Tube.
- Pre-equilibrate the silica fleece in the DNA Binding Column by adding 250 µL **Buffer GL** to the Column before applying sample. Centrifuge for 1 min at 10,000 x g and discard the flowthrough .

250 µL Buffer GL
10,000 x g 1 min

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③**To bind DNA**:

- Insert the DNA Binding Column into the Collection Tube.
- Transfer the sample from Step① into the Column.
- Insert the DNA Binding Column assembly into a standard tabletop microcentrifuge.
- Centrifuge for at 10,000 x g.

▲The maximum volume of the column reservoir is750µL. For sample volumes of mor than 750µL, simply load and spin again.

10,000 x g 1 min

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④**To wash the preparation:**

- Add 500µL of **Buffer GW2** to the upper reservoir of the Column.
- Centrifuge for 30 - 60 s at 10,000 x g and discard the flowthrough.

500µL Buffer GW2
10,000 x g 1 min

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⑤**To wash the preparation:**

- Combine the DNA binding column with the Collection Tube.
- Add 700µL of **Buffer GW2** to the upper reservoir of the Column.
- Centrifuge 1 min at 10,000×g and discard the flowthrough.

700µL Buffer GW2
10,000 x g 1 min

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⑥**After discarding the flowthrough liquid:**

- Centrifuge the DNA binding column for additional1 min at full speed.
- Discard the Collection Tube.

▲ The extra centrifugation time ensures removal of residual Wash Buffer.

full speed
1 min

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⑦**To elute the DNA**

- Insert the DNA binding column into a clean, sterile 1.5 ml Microcentrifuge tube.
- Add 10-25 µL prewarmed Buffer GE to the upper reservoir of the DNA binding column.
- Centrifuge the tube assembly for 1 min at 10,000×g 1min.

▲ Elution with 10µL (instead of 25µL) increases the final DNA concentration in the eluate , but also decreases the overall DNA yield.

▲ Warm up the Elution Buffer to 70℃ increases the final DNA concentration in the eluate.

10,000 x g
1 min

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Kit contents

Genorise Gel Extraction Kit PCR Clean Kit	Cat.NO.101081
DNA binding columns	200
Collection Tubes	200
1.5 ml microfuge tubes	200
Buffer GL 【*Optional steps*】	55ml
Buffer GP （PCR Clean）	100ml
Buffer GGA（Gel Extraction）	100ml
Buffer GGB（Gel Extraction）	60ml
Buffer GW2	72ml+178ml ethanol
Buffer GE	20ml

All buffer store at RT.

1. Introduction

The Purification system, designed for rapid DNA cleanup, includes:

- For direct purification of double- or single-stranded PCR products (100 bp–10 kb) from amplification reactions and DNA cleanup from other enzymatic reactions.
- For extraction of DNA fragments (70 bp – 10 kb) from standard, or low-melt agarose gels in TAE (Tris•acetate/EDTA) or TBE (Tris•borate/ EDTA) buffer and DNA cleanup from enzymatic reactions.

2. Protocol

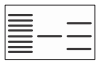
This protocol is designed to extract and purify DNA of 70 bp to 10 kb from standard or low-melt agarose gels in TAE or TBE buffer. Up to 400 mg agarose can be processed per spin column. This kit can also be used for DNA clean-up from enzymatic reactions.For DNA clean-up from enzymatic reactions using this protocol, add 3 volumes of **Buffer GP** and 1 volume of **isopropanol** to the reaction, mix, and proceed with step 6 of the protocol. Alternatively, use the Min Elute Reaction Clean-up Kit.

Important points before starting

- Add ethanol (95-100%) to Buffer GW2 before use (see bottle label for volume).

2.1 Protocol for DNA extraction from agarose gels

① Ⅰ. **Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.**



- Minimize the size of the gel slice by removing extra agarose.

Ⅱ. **Weigh the gel slice in a colorless tube. Add 3 volumes of Buffer GGA to 1 volume of gel (100 mg ~ 100μL)**

- For example, add 300 μL of **Buffer GGA** to each 100 mg of gel.
- For >2% agaros gels, add 6 volumes of **Buffer GGA**.
- The maximum amount of gel slice per column is 400 mg; for gel slices >400 mg use more than one column.

300μL Buffer GGA/100 mg Gel

Ⅲ. **Incubate at 55 -65°C for 10 min (or until the gel slice has Completely dissolved).To help dissolve gel, mix by vortexing the tube every 2-3 min during the incubation.**

▲Solubilize agarose completely. For >2% gels, increase incubation time.

Ⅳ.**Add Buffer GGB 0.5 volume of GGA to the sample and mix.**

- For example, if the agarose gel slice is 100 mg, add 300 μL **Buffer GGA**, Then add 150μL **Buffer GGB.**
- **Buffer GGB** volume= 0.5 volume **Buffer GGA.**