



**Cat.NO. GERC-101081-500**













Store the kit at 15 to 25°C

# **Genorise Gel DNA Purification Mini Kit PCR Clean Kit 500**

For Research Use only

## PCR clean-up, Gel extraction

### Protocol-at-a-glance

	PCR clean-up	Gel extraction
<b>1 PCR clean-up:</b> Adjust binding condition  <b>Gel extraction:</b> Excise DNA fragment / Solubilize gel slice	 100 $\mu$ L PCR 300 $\mu$ L Buffer GGA 150 $\mu$ L Buffer GGB  (Or 300 $\mu$ L Buffer GP)	  100 mg gel 300 $\mu$ L Buffer GGA 55°C 5-10 min 150 $\mu$ L Buffer GGB
<b>2 【Optional steps】</b>  Prepare The Column	 	250 $\mu$ L Buffer GL  10,000 x g 1 min
<b>3 Bind DNA</b>	 	10,000 x g 1 min
<b>4 Wash silica membrane</b> <b>5 Wash silica membrane</b>	 	500 $\mu$ L Buffer GW2 700 $\mu$ L Buffer GW2 10,000 x g 1 min
<b>6 Dry silica membrane</b>		10,000 x g 1 min
<b>7 Elute DNA</b>	 	10–25 $\mu$ L 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 100  $\mu$ L, add 300  $\mu$ L Buffer GGA, Add 150  $\mu$ L Buffer GGB
- mix, proceed with step 2.1 ② (page 6) of the protocol.

### @USE Buffer GP

- add 3 volumes of Buffer GP  
For example, if the PCR product is 100  $\mu$ L, add 300  $\mu$ L Buffer GP.
- mix, proceed with step 2.1 ② (page 6) of the protocol.

## 3. Troubleshooting

Problem	Possible cause and suggestions
<b>Incomplete Lysis of Agarose Slices</b>	<b>Time and temperature</b> • 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.
<b>Low DNA yield</b>	<b>Reagents not prepared properly</b> • Add indicated volume of 95–100% ethanol to Buffer GW2 Concentrate and mix well before use.
	<b>Incompletely dissolved gel slice</b> • 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.
	<b>Insufficient drying of the silica membrane</b> • 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.
	<b>Not enough elution buffer</b> • Especially when larger amounts of DNA (>5 $\mu$ g) are bound, increase elution buffer volume up to 50 $\mu$ L.
	<b>Isolation of large DNA fragments</b> • 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  $\mu$ L **Buffer GL** to the Column before applying sample. Centrifuge for 1 min at 10,000 x g and discard the flowthrough.



250  $\mu$ L Buffer GL  
10,000 x g 1 min

③ **To bind DNA:**

- Insert the DNA Binding Column into the Collection Tube.
- Transfer the sample from Step ① 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 is 750  $\mu$ L. For sample volumes of more than 750  $\mu$ L, simply load and spin again.



10,000 x g 1 min

④ **To wash the preparation:**

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



500  $\mu$ L Buffer GW2  
10,000 x g 1 min

⑤ **To wash the preparation:**

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



700  $\mu$ L Buffer GW2  
10,000 x g 1 min

⑥ **After discarding the flowthrough liquid:**

- Centrifuge the DNA binding column for additional 1 min at full speed.
- Discard the Collection Tube.
- ▲ The extra centrifugation time ensures removal of residual Wash Buffer.



full speed  
1 min

⑦ **To elute the DNA**

- Insert the DNA binding column into a clean, sterile 1.5 ml Microcentrifuge tube.
- Add 10-25  $\mu$ L prewarmed Buffer GE to the upper reservoir of the DNA binding column.
- Centrifuge the tube assembly for 1 min at 10,000 x g 1 min.
- ▲ Elution with 10  $\mu$ L (instead of 25  $\mu$ L) increases the final DNA concentration in the eluate, but also decreases the overall DNA yield.
- ▲ Warm up the Elution Buffer to 70  $^{\circ}$ C 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	GERC-101081-500
DNA binding columns	500
Collection Tubes	500
1.5 ml microfuge tubes	500
Buffer GL <b>【Optional steps】</b>	130ml
Buffer GP (PCR Clean)	100ml $\times$ 3
Buffer GGA (Gel Extraction)	100ml $\times$ 3
Buffer GGB (Gel Extraction)	75ml $\times$ 2
Buffer GW2	38ml +92ml ethanol $\times$ 5
Buffer GE	50ml

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% agarose 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**.