Contents

Kit Contents

Catalog no.	106-050
Spin column C132	50
Collection tube C947	50
Tune-up Buffer (UP Buffer)	15 ml
Gel Dissolving Buffer (GD buffer)	2 x 50 ml
Washing Buffer A concentrate (WA Buffer)	10 ml
DNA Elution Buffer (DE Buffer)	10 ml
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Product Description

GTpure™ Gel/PCR Purification Kit is designed for purification of DNA from both agarose gel and PCR reaction mixtures. Agarose gel slab are melted in the presence of the binding buffer (GD buffer). DNA from agarose gel, restriction digestions, enzyme modification reactions or PCR mix binds preferably onto the silica membranes of the spin column after the addition of isopropanol. Contaminants are washed away in a subsequent washing step. A low-salt elution buffer (DE buffer) is provided for efficient elution of the pure DNA from the spin column.

Storage

GTpure™ Gel/PCR Purification Kit should be kept dry at room temperature (15℃ – 25℃).The kit can be stored for up to 2 years.

Use Limitations

GTpure™ Gel/PCR Purification Kit is designed and manufactured for research purpose only.

Specifications

Maximum sample size	**400 mg (agarose gel) 400 µl (PCR mixture)**
Agarose gel buffer	**TAE or TBE buffer**
Agarose gel type	**Standard or low melting**
Maximum column reservoir	**800 µl**
Maximum DNA binding capacity	**12 µg**
Minimum elution volume	**30 µl**
Typical DNA percentage recovery 100 bp 1 kb 5 kb	** 70-80 % 80-90 % 75-85%**
Downstream applications	**Sequencing, cloning, ligation, restriction analysis/digestion/enzyme mapping, and PCR amplification**

Reagents and Consumables Supplied by Users

- **Equipments and consumables**
  - **Microcentrifuge**
  - **Scalpel or razor blade**
  - **56°C incubator or water bath**
  - **Sterile 1.5 ml microcentrifuge tubes**
  - **Pipette tips**
  - **Long wavelength UV light**
- **Reagents**
  - **Ethanol (96 – 100%)**
  - **Isopropanol (99-100%)**

Notes Before Using GTpure™ Gel/PCR Purification Kit

- Precipitates may form in GD buffer under low temperature. Re-dissolve any precipitates by incubating the buffer at 37 ℃ in the shortest possible time.
- All protocol steps are carried out at room temperature unless further specified.
- All centrifugation steps are carried out at *16,000 x g* unless further specified.
- Add pure ethanol (96%-100%) to **WA** Buffer as indicated on the bottle label.

Pack size	WA concentrate (ml)	Ethanol (ml)	Final volume (ml)
50	10	40	50

Protocols

Protocol A: Gel DNA extraction

1. **Using a scalpel or razor blade, excise the desired DNA fragment from agarose gel under long wavelength (320 nm) UV illumination.**

   - Minimize the size of the gel slab by removing extra gel slice.
   - Using long wavelength UV illumination has a lower chance of DNA cross-linking compared to short wavelength UV illumination.

2. **Weigh the gel slice in a 1.5 ml microcentrifuge tube (user provided).**

   - For gel slice > 400 mg, divide the slice into <400 mg slabs in separate 1.5-ml microcentrifuge tubes.
   - To account for an increase in reagent volume for 1.5-2% gel slice, divide the gel slice into <200 mg slabs in separate 1.5-ml microcentrifuge tubes or use larger tubes.

3. **Add 3 gel volumes of GD Buffer to 1 volume of 1% agarose gel (assuming 100 mg ~ 100 µl). For 2% agarose gel, add 6 volumes of GD Buffer.**

   - Add 300 µl GD Buffer to 100 mg 1% gel slice. For 100 mg 2% gel slice, add 600 µl GD Buffer. Proportionally adjust the volume of GD Buffer according to the gel % when using 1-2% agarose gel.

4. **Incubate at 56℃ until the gel slice is completely dissolved (usually < 10 min). Mix the tube contents by vortexing every 2-3 min. Make sure that the gel slice is completely dissolved before proceeding to the next step.**

   - For 2% gel slice, longer incubation time might be necessary for complete dissolution. Incomplete dissolution leads to lower yield.

5. **Add 2 gel volumes of isopropanol (user provided) to 1 volume of 1% agarose gel (100 mg ~ 100 µl). For 2% agarose gel, add 4 volumes isopropanol, and mix well.**

   - Add 200 µl isopropanol to 100 mg 1% gel slice. For 100 mg 2% gel slice, add 400 µl isopropanol. Proportionally adjust the volume of isopropanol according to the gel % when using 1-2% agarose gel.

6. **Insert a spin column C132 into a collection tube.**

7. **Add 250 µl UP Buffer to the spin column.**

8. **Centrifuge for 60 sec. Discard the flow-through and reuse the collection tube.**

9. **Apply up to 800 µl the sample to the spin column and centrifuge at *16,000 x g* for 30 sec.**

10. Discard the flow-through. Reuse the collection tube.

- For sample volume > 800 µl, apply up to 800 µl sample to the spin column, spin, discard flow through and repeat steps 9 and 10 until all sample is loaded.

11. Add 700 µl WA Buffer to the spin column and centrifuge at *16,000 x g* for 30 sec.

- Make sure that ethanol has been added to WA Buffer.

12. Discard the flow-through. Reuse the collection tube.

13. Centrifuge at 16,000 *x g* for an additional 1 min.

- Additional centrifugation ensures the removal of residual ethanol from the membrane. Any remaining ethanol can inhibit downstream applications.

14. Insert the spin column into a sterile 1.5-ml microcentrifuge tube (user provided).

15. Add 30 – 50 µl DE Buffer directly to the center of the spin column for maximal yield.

- 50 µl of DE Buffer would result in optimal recovery of DNA. To obtain higher eluted DNA concentration, use less DE Buffer, 30 µl for example.
- DE Buffer does not contain EDTA, therefore it does not inhibit subsequent enzymatic reactions.

16. Stand at room temperature for 1 min and centrifuge at 16,000 *x g* for 1 min.

- The eluted DNA is ready for downstream applications. For long-term storage, keep DNA at –20 ℃.

Protocol B: PCR product purification

1. **Insert a spin column C132 to a collection tube.**

2. **Add 250 µl UP Buffer to the spin column.**

3. **Centrifuge for 60 sec. Discard the flow-through and reuse the collection tube.**

4. **Add 3 volumes of GD Buffer to 1 volume of PCR sample. Use up to 400 µl PCR sample for each preparation**

   - For example, 300 µl GD Buffer to 100 µl PCR sample.

5. **Add 2 volume of isopropanol (user provided) to the sample and mix well.**

   - For example, 200 µl isopropanol to 100 µl PCR sample.

6. **Apply up to 800 µl the sample mix to the spin column and centrifuge at *16,000 x g* for 30 sec.**

7. **Discard the flow-through. Reuse the collection tube.**

   - For sample volume > 800 µl, apply up to 800 µl sample to the spin column, spin, discard flow through and repeat steps 6 and 7 until all sample is loaded.

8. **Add 700 µl WA Buffer to the spin column and centrifuge at *16,000 x g* for 30 sec.**

   - Make sure that ethanol has been added to WA Buffer.

9. **Discard the flow-through. Reuse the collection tube.**

10. **Centrifuge at *16, 000 x g* for an additional 1 min.**

    - Additional centrifugation ensures the removal of residual ethanol from the membrane. Any remaining ethanol can inhibit downstream applications.

11. **Insert the spin column into a sterile 1.5-ml microcentrifuge tube (user provided).**

12. **Add 30 – 50 µl DE Buffer directly to the center of the spin column for maximal yield.**

    - 50 µl of DE Buffer would result in optimal recovery of DNA. If higher eluted DNA concentration is needed, use a less DE Buffer, 30 µl for example.
    - DE Buffer does not contain EDTA, which may inhibit subsequent enzymatic reactions.

13. **Stand for 1 min and centrifuge at *16,000 x g* for 1 min.**

    - The eluted DNA is ready for downstream applications. For long-term storage, keep DNA at -20C.

Troubleshooting Guide

	Comments and Suggestions	
Section I: Low or no yield	**Not enough GD Buffer**	Ensure that the correct ratio of GD buffer to gel volume for a particular gel % is used in gel extraction protocol.
	Omission or not enough isopropanol	Ensure that the correct ratio of isopropanol to gel volume for a particular gel % is used in gel extraction protocol. Make sure the ratio of isopropanol to PCR product is 2:1.
	Insufficient mixing of isopropanol and sample mix	Mix the sample thoroughly after the addition of isopropanol. Vortex for 10 sec before applying the sample to the spin column.
	Omission of ethanol in WA Buffer	Ensure that the final concentration of ethanol in WA Buffer is 80%.
	Improper DE Buffer dispensing	Dispense DE Buffer directly onto the center of the membrane in the spin column
	Elution with distilled water	DE Buffer, pH 8.5, is recommended for DNA elution. If sterile distilled water is used instead, ensure that the pH of the water is ≥ 7.0. Elution efficiency drops drastically when pH < 7.0.
Section II: Poor performance in subsequent applications	**Residual ethanol in eluted DNA**	Ensure that the additional spinning step (step 11, protocol A; step 12, protocol B) is performed. To rescue eluted DNA contaminated with ethanol, perform ethanol precipitation, dry the DNA pellet thoroughly and dissolve in DE buffer. Alternatively, purify the eluted DNA using Protocol B of this manual. Prolong the centrifugation time in step 10 of protocol B to 2 min to remove residual ethanol.

Section II: Poor Performance in downstream applications	Comments and Suggestions	
	Carryover of salt in eluted DNA	Ensure that the WA buffer washing step (step 9, protocol A; step 8, protocol B) is performed. If salt contaminants are suspected to originate from the sample material, repeat the washing step once by adding 500 µl WA buffer. To rescue eluted DNA contaminated with salt, perform ethanol precipitation, dry the DNA pellet thoroughly and dissolve in DE buffer. Alternatively, purify the eluted DNA using Protocol B of this manual.
	EDTA in user-prepared elution buffer	EDTA in user-prepared elution buffer can inhibit downstream applications. DE buffer, which does not contain EDTA, is recommended for DNA elution.