



## PowerPrep™ Express Gel Extraction Kits

Catalog No. NP100012 (11456-019) 50 Reactions  
 Catalog No. NP100013 (11456-027) 250 Reactions  
 Catalog No. NP100014 (11457-017) 150 Reactions  
 Store at RT

### Overview and Intended Use

The PowerPrep™ Express Gel Extraction kits are designed to purify DNA fragments from agarose gels. The Express Gel Extraction Kits use spin cartridges containing silica membranes to capture and purify the DNA fragment. The Matrix Gel Extraction Kit uses silica resin, provided as slurry, to capture and purify the DNA fragment. In both systems, the agarose gel is dissolved by sodium perchlorate and the DNA is adsorbed onto the silica support (1). Adsorption to silica is influenced by buffer composition and temperature. Agarose and electrophoresis buffers are removed with alcohol-containing wash buffers. Since the DNA is eluted at high concentration in TE Buffer, no precipitation is necessary. All centrifugations are brief, allowing completion of the protocol in 25 to 30 min. The Gel Extraction Kits contain Gel Solubilization Buffer with a TBE-solubilizer. This enables extraction of DNA fragments from TAE or TBE buffer-containing agarose gels without additional solutions or modifications to the protocol. Fragments can be extracted from both low-melting point and high-melting-point agarose. DNA purified with these kits is of high quality and is suitable for a variety of applications, including automated fluorescent DNA sequencing, manual DNA sequencing, amplification reactions, *in vitro* transcription, restriction mapping, cloning and labeling.

- DNA fragments from 40 bp to 10 kb can be extracted from gels.
- Using the Express System, up to 15 µg of DNA can be purified with one spin cartridge.
- Using the Matrix System, up to 7.5 µg of DNA can be purified with 10 µl of Silica Resin.

### References

1. Vogelstein, B. and Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* 76, 615.

### Safety and Use Statement

This product contains hazardous reagents. It is the end-user's responsibility to consult the applicable MSDS(s) before using this product. Disposal of waste organics, acids, bases and radioactive materials must comply with all appropriate federal, state and local regulations. If you have any questions concerning the hazards associated with this product, please call OriGene at (888) 267-4436

All biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of potentially infectious or hazardous agents. This product is authorized for laboratory research use only. The product has not been qualified or found safe and effective for any human or animal diagnostic application. Uses other than the labeled intended use may be a violation of applicable law.

### Components included with this kit:

Component	EXPRESS Kits		MATRIX Kit
	NP100012	NP100013	NP100014
# Reactions	50	250	150
Gel Solubilization Buffer	2 x 75 mL	450 mL	100 mL
Wash Buffer	11 mL	55 mL	30 mL
TE Buffer	15 mL	15 mL	15 mL
Spin Cartridges, Wash Tubes and Recovery Tubes	50 each	50 each	—
Silica Resin (Matrix kit)	—	—	1.5 mL

### Additional Materials Required

- 95-100% ethanol
- Microcentrifuge capable of reaching  $\geq 12,000 \times g$
- 1.5- or 5-mL polypropylene tubes
- 50°C water bath or heat block
- 65 to 70°C water bath or heat block (for Express kits)

### Storage Conditions

Store all components at Room Temperature.

### Advance Preparations

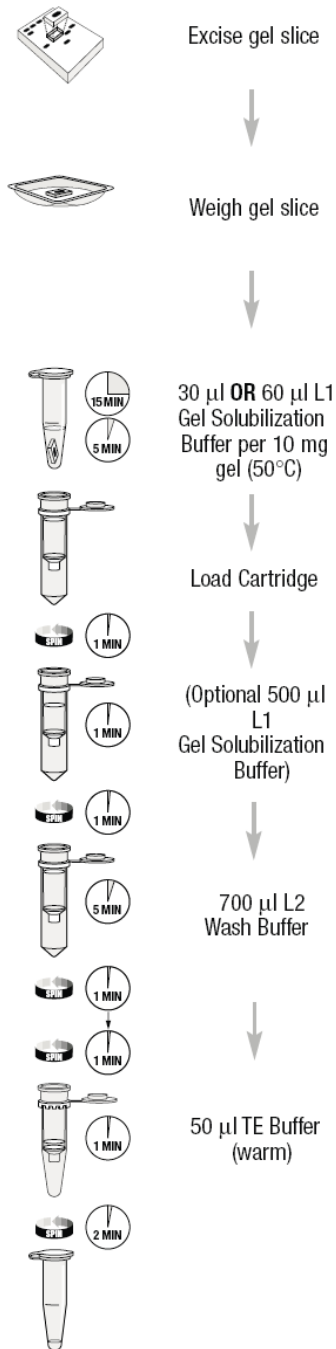
Add ethanol (EtOH) to Wash Buffer according to the following instructions.

Volume of Wash Buffer	Volume of EtOH to add to Wash Buffer
12 mL	30 mL
55 mL	140 mL
95 mL	235 mL

These instructions are also on the label of the bottle. Mix well. Place a mark on the label to indicate that ethanol has been added.

### Critical Parameters

- Use volumes, temperatures, incubation times and centrifugations precisely as indicated in the protocols.
- Supercoiled DNA cannot be gel purified.
- Elutions are performed at elevated temperatures to improve DNA recovery.
- Complete dissolution of the agarose gel slice is critical. Once the gel slice appears dissolved, an additional 5 min. incubation period at 50°C is required.



## Usage Notes – Microcentrifuge Protocol

Perform all centrifugations at room temperature.

### Before beginning:

- Preheat an aliquot of **TE Buffer** to 65 to 70°C.
- Equilibrate a water bath or heat block to 50°C.
- Verify that ethanol has been added to **Wash Buffer** (See Advance Preparations).

**1. Gel Slice Excision:** Cut the area of gel containing the DNA fragment using a clean, sharp blade. Minimize the amount of surrounding agarose excised with the fragment.

**2. Gel Slice Weighing:** Weigh the gel slice.

a. For  $\leq 2\%$  agarose gels, place up to 400 mg of gel into a 1.5-mL polypropylene tube. Divide gel slices exceeding 400 mg among additional tubes. Add 30  $\mu\text{L}$  of Gel Solubilization Buffer for every 10 mg of gel.

- OR -

b. For  $\geq 2\%$  agarose gels, place up to 400 mg of gel into a 5-mL polypropylene tube. Divide gel slices exceeding 400 mg among additional tubes. Add 60  $\mu\text{L}$  of Gel Solubilization Buffer for every 10 mg of gel.

**3. Gel Solubilization:** Incubate at 50°C for 15 min. Mix every 3 min to ensure gel dissolution. After gel slice appears dissolved, incubate for 5 min longer.

NOTE: High concentration gels, i.e.,  $\geq 2\%$  agarose, or large gel slices may take longer to dissolve.

**4. Cartridge Loading:** Place a cartridge into a 2-mL wash tube. Pipet the mixture from step 3 into the cartridge and centrifuge in a microcentrifuge at  $\geq 12,000 \times g$  for 1 min. Discard the flow-through.

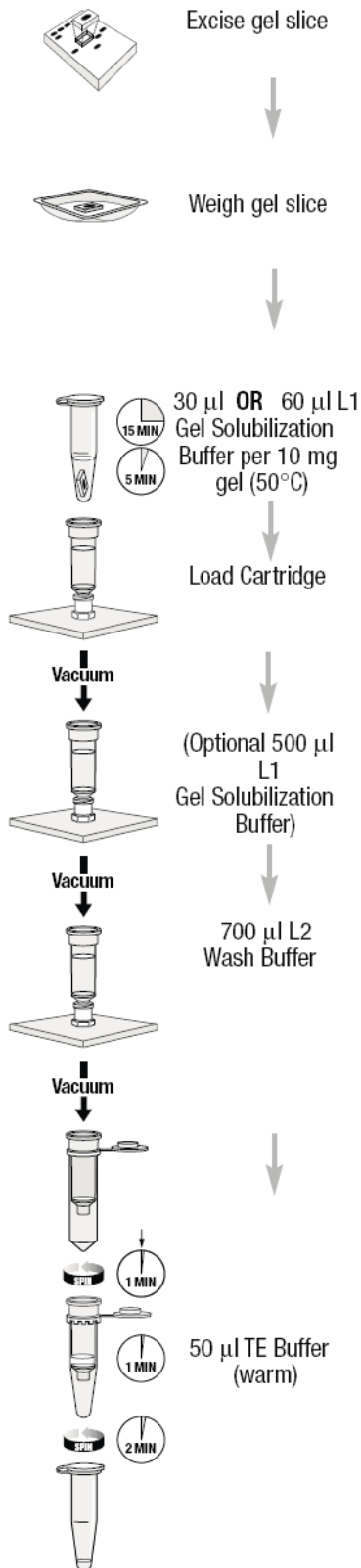
NOTE: Load no more than 400 mg agarose per cartridge.

**5. (Optional Cartridge Wash):** Place the cartridge back into the 2-mL wash tube. Add 500  $\mu\text{L}$  Gel Solubilization Buffer to the cartridge. Incubate at room temperature for 1 min, then centrifuge at  $\sim \geq 12,000 \times g$  for 1 min. Discard the flow-through. NOTE: This wash is recommended when  $\geq 250$  mg of gel has been loaded onto a cartridge or when higher purity is required for applications such as DNA sequencing or *in vitro* transcription.

**6. Cartridge Wash:** Place the cartridge back into the 2-mL wash tube. Add 700  $\mu\text{L}$  of Wash Buffer (containing ethanol) to the cartridge and incubate for 5 min at room temperature. Centrifuge at  $\geq 12,000 \times g$  for 1 min. Discard the flow-through. Centrifuge again for 1 min to remove residual wash buffer.

**7. DNA Elution:** Place the cartridge into a 1.5-mL recovery tube (supplied). Add 50  $\mu\text{L}$  of warm TE Buffer directly to the center of the cartridge. Incubate for 1 min at room temperature, then centrifuge at  $\geq 12,000 \times g$  for 2 min.

NOTE: Room temperature TE Buffer may be used for elution, but optimal recovery is obtained with TE warmed to 65 to 70°C.



## Usage Notes – Vacuum Manifold Protocol

**Perform all centrifugations at room temperature.**

### Before beginning:

- Preheat an aliquot of **TE Buffer** to 65 to 70°C.
- Equilibrate a water bath or heat block to 50°C.
- Verify that ethanol has been added to the **Wash Buffer** (See Advance Preparations).

**1. Gel Slice Excision:** Cut the area of gel containing the DNA fragment using a clean, sharp blade. Minimize the amount of surrounding agarose excised with the fragment.

**2. Gel Slice Weighing:** Weigh the gel slice.

- For  $\leq 2\%$  agarose gels, place up to 400 mg of gel into a 1.5-mL polypropylene tube. Divide gel slices exceeding 400 mg among additional tubes. Add 30  $\mu\text{L}$  of Gel Solubilization Buffer for every 10 mg of gel.

- OR -

- For  $\geq 2\%$  agarose gels, place up to 400 mg of gel into a 5-mL polypropylene tube. Divide gel slices exceeding 400 mg among additional tubes. Add 60  $\mu\text{L}$  of Gel Solubilization Buffer for every 10 mg of gel.

**3. Gel Solubilization:** Incubate at 50°C for 15 min. Mix every 3 min to ensure gel dissolution. After gel slice appears dissolved, incubate for 5 min longer.

NOTE: High concentration gels, *i.e.*,  $\geq 2\%$  agarose, or large gel slices may take longer to dissolve.

**4. Vacuum Manifold Preparation:** Attach the vacuum manifold to a vacuum source. Attach a cartridge to a luer extension on the vacuum manifold.

**5. Cartridge Loading:** Load the sample from step 3 into the cartridge. Apply vacuum until all liquid goes through the cartridge, and then turn off the vacuum source.

NOTE: Load no more than 400 mg agarose per cartridge.

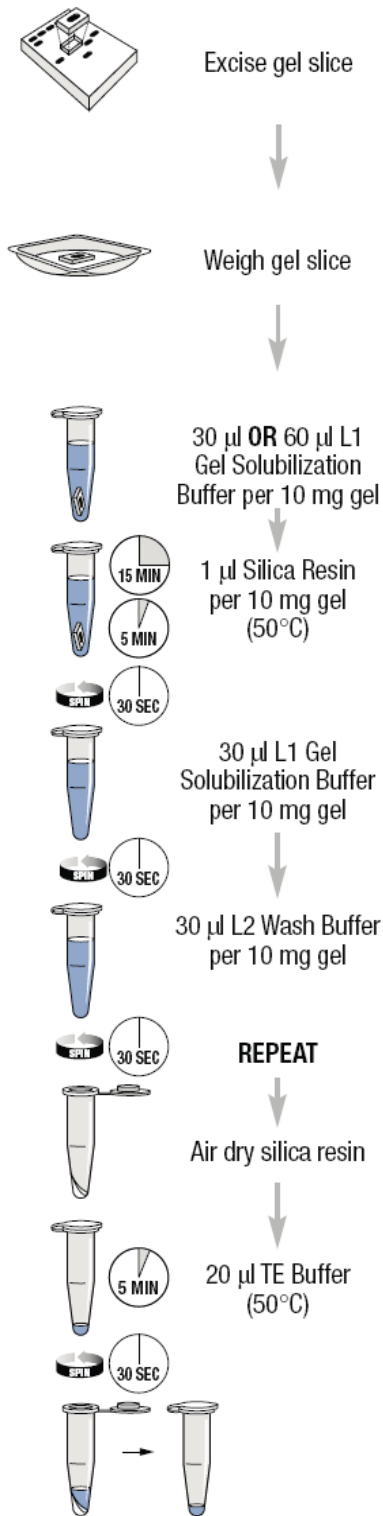
**6. (Optional Cartridge Wash):** Add 500  $\mu\text{L}$  Gel Solubilization Buffer to the cartridge. Apply vacuum until all liquid goes through the cartridge, and then turn off the vacuum source.

NOTE: This wash is recommended when  $\geq 250$  mg of gel has been loaded onto a cartridge or when higher purity is required for applications such as DNA sequencing or *in vitro* transcription.

**7. Cartridge Wash:** Add 700  $\mu\text{L}$  of Wash Buffer (containing ethanol) to the cartridge. Apply vacuum until all liquid goes through the cartridge and then turn off the vacuum source. Remove the cartridge from the manifold and place into a 2-mL wash tube. Centrifuge at  $\geq 12,000 \times g$  for 1 min to remove all residual wash buffer.

**8. DNA Elution:** Place the cartridge into a 1.5-mL recovery tube (supplied). Add 50  $\mu\text{L}$  of warm TE Buffer directly to the center of the cartridge. Incubate for 1 min at room temperature, then centrifuge at  $\geq 12,000 \times g$  for 2 min.

NOTE: Room temperature TE may be used for elution, but optimal recovery is obtained with TE warmed to 65 to 70°C.



## Usage Notes – Gel Extraction Kit

**Perform all centrifugations at room temperature.**

### Before beginning:

- a. Preheat an aliquot of TE Buffer to 65 to 70°C.
  - b. Verify that RNase A has been added to Cell Suspension Buffer and that no precipitate has formed in Cell Lysis Solution.
  - c. Verify that ethanol has been added to Wash Buffer and Optional Wash Buffer (See Advance Preparations).
1. Gel Slice Excision: Cut the area of gel containing the DNA fragment using a clean, sharp blade. Minimize the amount of surrounding agarose excised with the fragment.
  2. Gel Slice Weighing: Weigh the gel slice.
    - a. For  $\leq 2\%$  agarose gels, place up to 400 mg of gel into a 1.5-mL polypropylene tube. Divide gel slices exceeding 400 mg among additional tubes. Add 30  $\mu$ L of Gel Solubilization Buffer for every 10 mg of gel.
    - OR -
    - b. For  $\geq 2\%$  agarose gels, place up to 400 mg of gel into a 5-mL polypropylene tube. Divide gel slices exceeding 400 mg among additional tubes. Add 60  $\mu$ L of Gel Solubilization Buffer for every 10 mg of gel.
  3. Gel Solubilization and DNA Binding: Vortex the Silica Resin until it is thoroughly suspended. Add 1  $\mu$ L of Silica Resin for every 10 mg of gel. Vortex and incubate at 50°C for  $\geq 15$  min. Mix every 3 min to ensure gel dissolution. After gel slice appears dissolved, incubate for 5 min longer. NOTE: High concentration gels, *i.e.*,  $\geq 2\%$  agarose, or large gel slices may take longer to dissolve.
  4. DNA-Silica Resin Isolation: Centrifuge at  $\geq 12,000 \times g$  for 30 s. Carefully and thoroughly remove supernatant with a pipette and discard.
  5. High Salt Wash: Add 30  $\mu$ L of Gel Solubilization Buffer for every 10 mg of gel. Suspend resin by vortexing or by flicking the tube. Centrifuge at  $\geq 12,000 \times g$  for 30 s. Carefully and thoroughly remove supernatant with a pipette and discard.
  6. Low Salt Wash: Add 30  $\mu$ L of Wash Buffer (containing ethanol) for every 10 mg of gel. Suspend resin by vortexing or by flicking the tube. Centrifuge at  $\geq 12,000 \times g$  for 30 s. Carefully and thoroughly remove supernatant with a pipette and discard. Repeat step 6.
  7. Resin Drying: Air dry the silica resin to remove residual ethanol from the wash buffer. Do not overdry.
  8. DNA Elution: Add 20  $\mu$ L of TE Buffer and suspend the resin by vortexing or by flicking the tube. Incubate for 5 min at 50°C. Mix once during the incubation. Centrifuge at  $\geq 12,000 \times g$  for 30 s. Carefully pipet the supernatant into a fresh tube. If resin is visible in the sample, repeat the centrifugation and transfer the supernatant to a fresh tube.

**Appendix A  
Troubleshooting Guide**

Problem	Possible Cause	Suggested Solution
Low yield of DNA elution	Incorrect ratio of gel to Gel Solubilization Buffer	Ensure that correct volume of Gel Solubilization Buffer is added for every 10 mg of gel, e.g., 120 mg of 2% gel will require 360 $\mu$ L of Gel Solubilization Buffer.
	Incomplete solubilization of gel	Verify that the temperature of water bath or heat block is at 50°C.  Cut large gel slices into several pieces to speed dissolution.  Mix gel slice in Gel Solubilization Buffer every 3 min during the dissolution step.
	Elution temperature is low	<b>For Express Kit:</b> TE Buffer should be fully equilibrated to 65 to 70°C before elution.  <b>For Matrix Kit:</b> Verify that the temperature of water bath or heat block is at 50°C.
	DNA fragment is large	<b>For Matrix Kit:</b> Increase elution incubation to 15 min.  Increase elution temperature to 60°C.
	DNA is supercoiled	These systems are not designed to extract supercoiled plasmid DNA from agarose gels.
Inhibition of enzymatic reaction	Residual ethanol in the sample	<b>For Express Kit:</b> Discard wash buffer flow-through prior to the second centrifugation in step 6.  <b>For Matrix Kit:</b> Air dry silica resin pellet before adding TE Buffer to elute.
	Excess salt in the sample	<b>For Matrix Kit:</b> Thoroughly remove all of the last wash before drying resin.
	DNA contaminated with silica resin	<b>For Matrix Kit:</b> Centrifuge sample at $\geq 12,000 \times g$ for 1 min and carefully transfer the supernatant to a fresh tube.

**Technical Support**

For further technical assistance please contact us at (888) 267-4436 or by email at [techsupport@origene.com](mailto:techsupport@origene.com). Technical support and troubleshooting guides for these products can also be found on our website at [www.origene.com](http://www.origene.com).

**Related Products**

To see our full line of PowerPrep™ purification products visit our website at [www.origene.com](http://www.origene.com).