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PureLink® Quick Gel Extraction and PCR Purification Combo Kit

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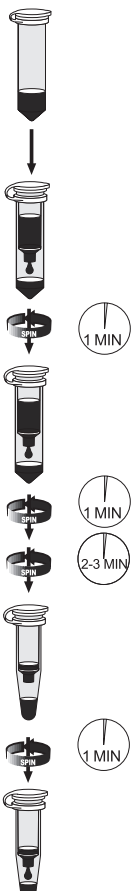
Purifying PCR Products



Before Starting. Add 96–100 % ethanol to Wash Buffer (W1) and isopropanol to Binding Buffer (B2) according to the labels on the bottles.

1. **Combine.** Add 4 volumes of Binding Buffer (B2) with isopropanol to 1 volume of a PCR sample (50–100 µL). Mix well.

2. **Load.** Pipet the sample into a PureLink® Clean-up Spin Column in a Wash Tube. Centrifuge the column at >10,000 × *g* for 1 minute. Discard the flow-through.

3. **Wash.** Re-insert the column into the Wash Tube and add 650 µL Wash Buffer (W1) with ethanol. Centrifuge the column at >10,000 × *g* for 1 minute.

4. **Remove ethanol.** Discard the flow-through and place the column in the same Wash Tube. Centrifuge the column at maximum speed for 2–3 minutes.

5. **Elute.** Place the column into a clean 1.7-mL Elution Tube. Add 50 µL Elution Buffer (E1) to the column. Incubate the column at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute.

6. **Store.** *The elution tube contains the purified PCR product.* Store the purified DNA at 4°C for immediate use or at –20°C for long-term storage.

Intended Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

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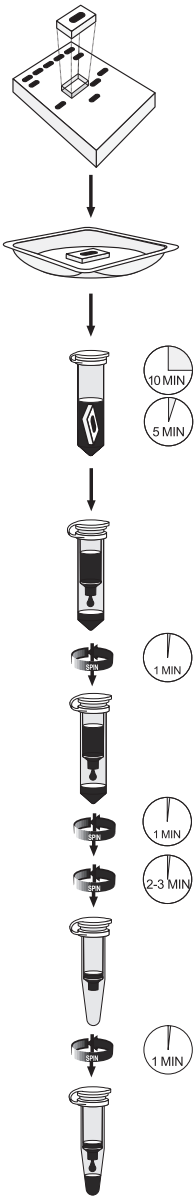
Purifying DNA from Gels Using a Centrifuge



Before Starting. Add ethanol to Wash Buffer (W1) according to the label on the bottle. Equilibrate a water bath or heat block to 50°C.

1. **Excise**. Use a clean, sharp razor blade to excise a minimal area of gel containing the DNA fragment of interest.

2. **Weigh.** Using a scale sensitive to 0.001 g, weigh the gel slice containing the DNA fragment.

3. **Solubilize.** Add Gel Solubilization Buffer (L3) to the excised gel in a tube as indicated in the table. Incubate the tube at 50°C for 10 minutes (or longer for large gel slices and high concentration gels), and invert the tube every 3 minutes. After the gel slice appears dissolved, incubate the tube for an additional 5 minutes. *(Optional)* Add 1 gel volume of isopropanol to the dissolved gel slice. Mix well.

Gel	Tube	Buffer L3 Volume
≤2% agarose	1.7-mL polypropylene	3:1
>2% agarose	5-mL polypropylene	6:1

4. **Load.** Pipet the dissolved gel piece onto a column inside a Wash Tube. Centrifuge the column at >12,000 × *g* for 1 minute. Discard the flow-through and place the column into the Wash Tube.

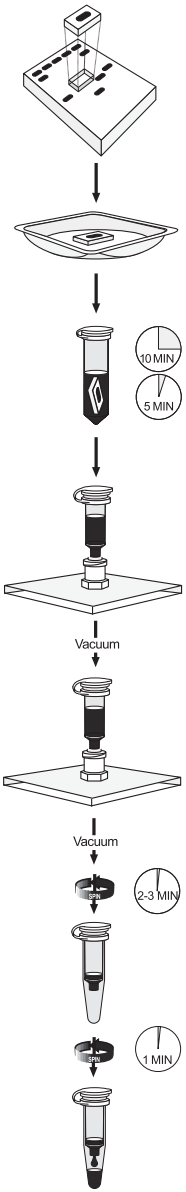
   Note: The column reservoir capacity is 850 µL. Use 1 column per 400 mg of agarose gel.

5. **Wash.** Add 500 µL Wash Buffer (W1) containing ethanol to the column. Centrifuge the column at >12,000 × *g* for 1 minute. Discard the flow-through and place the column into the Wash Tube. Centrifuge the column at maximum speed for 2–3 minutes. Discard the flow-through.

6. **Elute.** Place the column into a Recovery Tube. Add 50 µL Elution Buffer (E1) to the column. Incubate the tube for 1 minute at room temperature. Centrifuge the tube at >12,000 × *g* for 1 minute.

7. **Store.** *The elution tube contains the purified DNA.* Store the purified DNA at 4°C for immediate use or at –20°C for long-term storage.

Purifying DNA from Gels Using a Vacuum Manifold



Before Starting. Add ethanol to Wash Buffer (W1) according to the label on the bottle. Equilibrate a water bath or heat block to 50°C.

1. **Excise**. Use a clean, sharp razor blade to excise a minimal area of gel containing the DNA fragment of interest.

2. **Weigh.** Using a scale sensitive to 0.001 g, weigh the gel slice containing the DNA fragment.

3. **Solubilize.** Add Gel Solubilization Buffer (L3) to the excised gel in a tube as indicated in the table. Incubate the tube at 50°C for 10 minutes (or longer for large gel slices and high concentration gels), and invert the tube every 3 minutes. After the gel slice appears dissolved, incubate the tube for an additional 5 minutes. *(Optional)* Add 1 gel volume of isopropanol to the dissolved gel slice. Mix well.

Gel	Tube	Buffer L3 Volume
≤2% agarose	1.7-mL polypropylene	3:1
>2% agarose	5-mL polypropylene	6:1

4. **Load.** Remove the Wash Tube, and attach a column to the vacuum manifold. Pipet the dissolved gel mixture containing the DNA of interest onto the center of the column. Apply vacuum until all of the liquid passes through the column.

   Note: The column reservoir capacity is 850 µL. Use 1 column per 400 mg of agarose gel.

5. **Wash.** Add 500–700 µL Wash Buffer (W1) containing ethanol to the column. Apply vacuum until all of the liquid passes through the column. Place the column into a Wash Tube. Centrifuge the column at maximum speed for 2–3 minutes. Discard the flow-through.

6. **Elute.** Place the column into a Recovery Tube. Add 50 µL Elution Buffer (E1) to the column. Incubate the tube for 1 minute at room temperature. Centrifuge the tube at >12,000 × *g* for 1 minute.

7. **Store.** *The elution tube contains the purified DNA.* Store the purified DNA at 4°C for immediate use or at –20°C for long-term storage.

Troubleshooting

Problem	Solution
Low DNA yield	**PCR Purification:** • Check the amplicon on the gel to verify the PCR product prior to purification. • Always mix 1 volume of PCR (50–100 µL) with 4 volumes of Binding Buffer (B2). • Add 100% isopropanol to the Binding Buffer (B2) and 96–100% ethanol to Wash Buffer (W1). • Add Elution Buffer (E1) to the center of the column. Incubate the tube with Elution Buffer for 1 minute before centrifugation **Gel Extraction:** • Ensure that the correct volume of Gel Solubilization Buffer (L3) is added. • Verify that the temperature of water bath or heat block is 50°C. • Cut large gel slices into several pieces. • Mix the gel slice in the buffer every 3 minutes during the dissolution step. • Increase the incubation time for elution to >10 minutes. **Note:** This kit is not designed to purify supercoiled plasmid DNA.
Low $A_{260/230}$ ratio	**Gel Extraction:** • Do not get any buffer solution in the cap area of the tube. • Add a second wash step with Wash Buffer (W1): After washing the DNA with Wash Buffer (step 5): 1. Add another 500–700 µL Wash Buffer containing ethanol. 2. Centrifuge the tube at 12,000 × *g*. Discard the flow-through and return the column into the Wash Tube. 3. Centrifuge at the tube at maximum speed for 2–3 minutes.
Enzymatic reactions are inhibited	**For all Procedures:** To remove Wash Buffer, discard Wash Buffer flow-through from the Wash Tube. Place the column into the Wash Tube and centrifuge the column at >12,000 × *g* for 2–3 minutes to completely dry the column.

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