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GeneJET™ Gel Extraction Kit

USER GUIDE

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For Research Use Only. Not for use in diagnostic procedures.

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0012661 B (English)

Revision	Date	Description
B	24 April 2025	• Updated to the current document template, with associated updates to the limited license information, warranty, trademarks, and logos. • The contents and storage table was updated to include additional instructions for storage of purification columns.
A.0	17 October 2015	Baseline for revision history.

The information in this guide is subject to change without notice.

Contents

1 Product information

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The GeneJET™ Gel Extraction Kit is designed for rapid and efficient purification of DNA fragments from standard or low-melting point agarose gels run in either TAE or TBE buffer. The kit uses a proprietary silica-based membrane technology in the form of a convenient spin column. The kit can be used to purify DNA fragments from 25 bp to 20 kb in size. The recovery rates are up to 95% in a 100 bp–10 kb DNA fragment size range (see Figure 1). Each purification column has a binding capacity of up to 25 µg of DNA and can process up to 1 g of agarose gel.

The entire procedure takes 15 minutes and the isolated DNA is ready to use in all common downstream applications including ligation, restriction digestion, PCR, sequencing, and labeling.

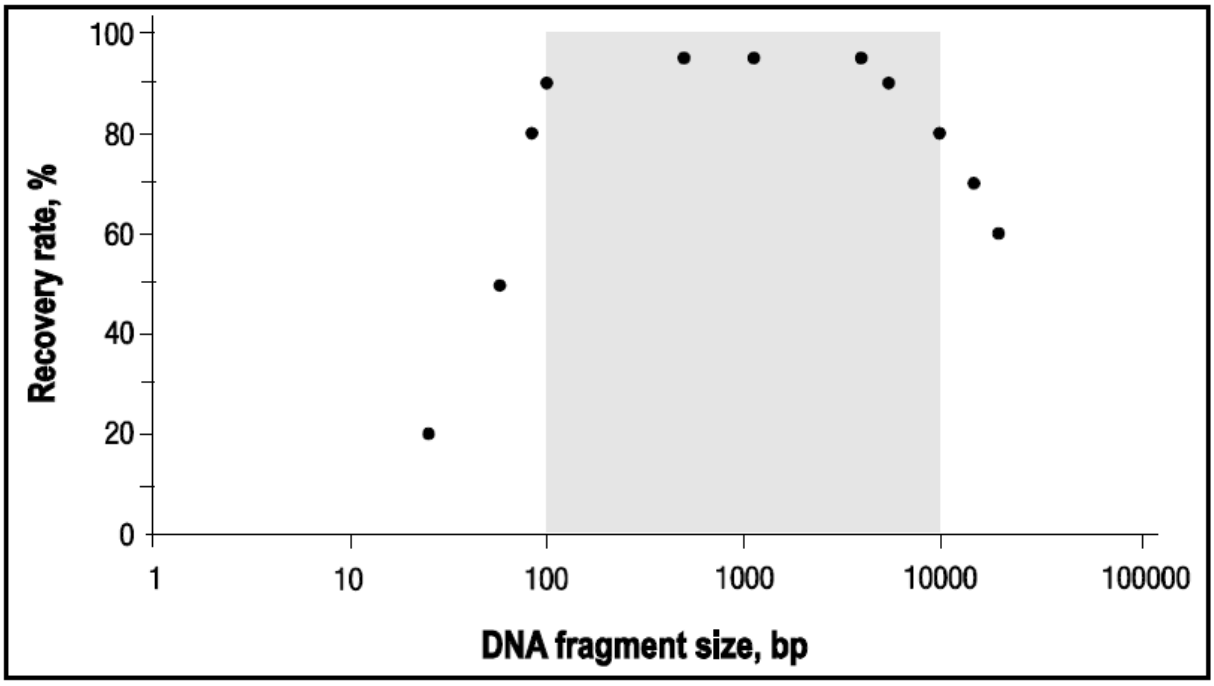

Figure 1 Recovery dependence on DNA fragment size

Technology overview

The DNA fragment of interest is excised from an agarose gel, placed in a microcentrifuge tube, solubilized in binding buffer, and applied to the column. The chaotropic agent in the binding buffer dissolves agarose, denatures proteins, and promotes DNA binding to the silica membrane in the column. As an added convenience, the binding buffer contains a color indicator that allows for easy monitoring of the solution pH for optimal DNA binding. Impurities are removed with a simple wash step. Purified DNA is then eluted from the column with the elution buffer. The recovered DNA is ready for use in downstream applications.

Contents and storage

Table 1 Components of the GeneJET™ Gel Extraction Kit

Component	Cat. No. K0691 (50 reactions)	Cat. No. K0692 (250 reactions)	Storage
Binding Buffer	30 mL	150 mL	15–25°C
Wash Buffer (concentrated)	9 mL	45 mL	
Elution Buffer (10 mM Tris-HCl, pH 8.5)	15 mL	30 mL	
GeneJET Purification Columns (preassembled with collection tubes)	50	250	15–25°C For better long-term performance store at 2°C to 8°C.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Adjustable micropipettor	**MLS**
Thermo heating-blocks or water bath (adjustable to 56°C)	**MLS**
Microcentrifuge of ≥12,000 × *g* for microcentrifuge tubes	**MLS**
Vacuum manifold	**MLS**
Microcentrifuge tubes (1.5 mL or 2 mL)	**MLS**
RNase-free pipette tips (sterile)	**MLS**
Disposable gloves	**MLS**

(continued)

Item	Source
Ethanol, 96–100% (molecular biology grade)	MLS
Isopropanol	MLS
3 M sodium acetate, pH 5.2 (May be necessary. See step 5 on page 7)	MLS

Procedural guidelines

IMPORTANT! Tightly seal the bag containing GeneJET™ Purification Columns after each use.

- Wear gloves when handling the Binding Buffer this solution contains irritants and is harmful if contacted with skin, inhaled, or swallowed.
- Do not reuse electrophoresis buffer when extracted DNA fragment will be used directly for sequencing.
- All purification steps should be carried out at room temperature.
- All centrifugation steps should be carried out at >12,000 × *g*.

Before first use of the kit

1. Add the indicated volume of ethanol (96–100%) to Wash Buffer (concentrated) before first use.

Component	Cat. No. K0691 (50 reactions)	Cat. No. K0692 (250 reactions)
Wash Buffer (concentrated)	9 mL	45 mL
Ethanol (96–100%)	45 mL	225 mL
Total volume	**54 mL**	**270 mL**

2. Mark the checkbox on the bottlecap to indicate that ethanol has been added to the bottle.

Before each use of the kit

1. Check Binding Buffer for precipitation before each use.
2. Redissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use.

2 Methods

Extract DNA from gel using centrifuge

Dissolve gel

1. Excise gel slice containing the DNA fragment using a clean scalpel or razor blade. Cut as close to the DNA as possible to minimize the gel volume.

   Note: If the purified fragment will be used for cloning reactions, avoid damaging the DNA through UV light exposure. Minimize UV exposure to a few seconds or keep the gel slice on a glass or plastic plate during UV illumination.

2. Place the gel slice into a preweighed 1.5 mL tube and weigh. Record the weight of the gel slice.

3. Add 1:1 volume of Binding Buffer to the gel slice (volume: weight).

   Note: For example, add 100 µL of Binding Buffer for every 100 mg of agarose gel.

   Note: For gels with an agarose content >2%, add 2:1 volumes of Binding Buffer to the gel slice.

4. Incubate the gel mixture at 50–60°C for 10 minutes or until the gel slice is completely dissolved. Mix the tube by inversion every few minutes to facilitate the melting process.

5. Ensure that the gel is completely dissolved. Vortex the gel mixture briefly before loading on the column.

   Note: Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is orange or violet, add 10 µL of 3 M sodium acetate, pH 5.2 solution, then mix. The color of the mix will become yellow.

6. *(Optional)* Use this step only when DNA fragment is ≤500 bp or >10kb long.

   - If the DNA fragment is ≤500 bp, add 1 gel volume of 100% isopropanol to the solubilized gel solution. Mix thoroughly.

   Note: For example, 100 µL of isopropanol should be added to 100 mg gel slice solubilized in 100 µL of Binding Buffer.

   - If the DNA fragment is >10 kb, add 1 gel volume of water to the solubilized gel solution. Mix thoroughly.

   Note: For example, 100 µL of water should be added to 100 mg gel slice solubilized in 100 µL of Binding Buffer.

Purify DNA

1. Transfer up to 800 µL of the solubilized gel solution (from step 5 or step 6) to the purification column.
2. Centrifuge at >12,000 × *g* for 1 minute. Discard the flow-through, then place the column back into the same collection tube.

   Note: If the total volume exceeds 800 µL, the solution can be added to the column in stages. After each application, centrifuge at >12,000 × *g* the column for 30–60 seconds, then discard the flow-through after each spin. Repeat until the entire volume has been applied to the column membrane. Do not exceed 1 g of total agarose gel per column.

3. *(Optional)* Use this additional binding step only if the purified DNA will be used for sequencing.
   - Add 100 µL of Binding Buffer to the purification column. Centrifuge at >12,000 × *g* for 1 minute. Discard the flow-through, then place the column back into the same collection tube.
4. Add 700 µL of Wash Buffer supplemented with ethanol (see "Before first use of the kit" on page 6) to the purification column.
5. Centrifuge at >12,000 × *g* for 1 minute. Discard the flow-through, then place the column back into the same collection tube.
6. Centrifuge the empty purification column at >12,000 × *g* for an additional 1 minute to completely remove residual wash buffer.

   IMPORTANT! This step is essential to avoid residual ethanol in the purified DNA solution. The presence of ethanol in the DNA sample may inhibit downstream enzymatic reactions.

7. Transfer the purification column into a clean 1.5 mL microcentrifuge tube.
8. Add 50 µL of Elution Buffer to the center of the purification column membrane. Centrifuge at > 12,000 × *g* for 1 minute. Discard the purification column.

   Note:
   - For low DNA amounts the elution volumes can be reduced to increase DNA concentration. An elution volume between 20–50 µL does not significantly reduce the DNA yield. However, elution volumes less than 10 µL are not recommended.
   - If DNA fragment is >10 kb, prewarm Elution Buffer to 65°C before applying to column.
   - If the elution volume is 10 µL and DNA amount is ≤5 µg, incubate column for 1 minute at room temperature before centrifugation.

Use the purified DNA immediately in downstream applications or store the purified DNA at -20°C.

Extract DNA from gel using vacuum manifolds

Dissolve gel

Excise a gel slice containing DNA fragment and completely dissolve it. See “Dissolve gel” on page 7.

Note: Do not exceed 1 g of total agarose gel per column.

Purify DNA

1. Prepare the vacuum manifold according to the supplier’s instructions.
2. Place the purification column(s) onto the manifold.
3. Transfer up to 800 µL of the solubilized gel solution (from step 5 or step 6) to the purification column.

   Note: If the total volume of solubilized gel solution exceeds 800 µL, the solution can be added to the column in stages. After each application, apply the vacuum and discard the flow-through. Repeat until the entire volume has been applied to the column membrane. Do not exceed 1 g of total agarose gel per column.
4. Apply the vacuum to draw the sample through the column. Switch off the vacuum after the entire sample has passed through the column.
5. *(Optional)* Use this additional binding step only if the purified DNA will be used for sequencing.
   - Add 100 µL of Binding Buffer to the purification column. Apply the vacuum to draw the sample through the column. Switch off the vacuum after the entire sample has passed through the column.
6. Add 700 µL of Wash Buffer supplemented with ethanol (see “Before first use of the kit” on page 6) to the purification column.
7. Apply the vacuum to draw the solution through the column. Switch off the vacuum after the solution has passed through the column. Place the column back into the same collection tube.
8. Centrifuge the empty purification column at >12,000 × *g* for an additional 1 minute to completely remove residual wash buffer.

   IMPORTANT! This step is essential to avoid residual ethanol in the purified DNA solution. The presence of ethanol in the DNA sample may inhibit downstream enzymatic reactions.
9. Transfer the purification column into a clean 1.5 mL microcentrifuge tube.

10. Add 50 µL of Elution Buffer to the center of the purification column membrane. Centrifuge at > 12,000 × *g* for 1 minute. Discard the purification column.

Note:

- For low DNA amounts the elution volumes can be reduced to increase DNA concentration. An elution volume between 20–50 µL does not significantly reduce the DNA yield. However, elution volumes less than 10 µL are not recommended.
- If DNA fragment is >10 kb, prewarm Elution Buffer to 65°C before applying to column.
- If the elution volume is 10 µL and DNA amount is ≤5 µg, incubate column for 1 minute at room temperature before centrifugation.

Use the purified DNA immediately in downstream applications or store the purified DNA at -20°C.

A Troubleshooting

Observation	Possible cause	Recommended action
Low DNA yield	Incomplete solubilization of the gel slice.	Verify that a 1:1 volume of Binding Buffer is added to a precisely weighted gel slice.
		Ensure that the gel slice is completely dissolved before applying to the purification column. A large amount of agarose or a gel slice with an agarose percentage >2% may require extra time to dissolve. In some cases, larger volumes of Binding Buffer and additional vortexing of the gel solution facilitate solubilization.
	Inefficient DNA binding.	Check the color of the solution after the gel slice is completely dissolved. A yellow color indicates an optimal pH for DNA binding. If the solution color is orange or violet, add 10 µL of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow.
	Inefficient membrane wash.	Ensure that recommended volume of ethanol was added to the Wash Buffer (concentrated) prior first use (see "Before first use of the kit" on page 6).
	Inefficient DNA elution.	Add the Elution Buffer directly to the center of the membrane and not to the side of the purification column.
		Use 20–50 µL of Elution Buffer and ensure that the volume completely covers the surface of the membrane.
		Increase the Elution Buffer volume twice or perform two elution cycles when purifying larger amounts of DNA (For example, >15 µg).
		In step 6, ensure all residual wash buffer is removed from the membrane. Longer centrifugation time (extra minute) can aid in removal of wash buffer.
DNA does not remain in an agarose gel well	Presence of residual ethanol.	In step 6, ensure all residual wash buffer is removed from the membrane. Longer centrifugation time can aid in removal of wash buffer.
Low quality sequencing results	Contamination from reused electrophoresis buffer.	If extracted DNA will be used directly for sequencing, freshly prepared electrophoresis buffers should be used both for gel preparation and for gel running.
Downstream applications are unsuccessful	Presence of residual ethanol.	In step 6 of "Purify DNA" on page 8 using centrifuge or step 8 of "Purify DNA" on page 9 using vacuum manifold, ensure all residual wash buffer is removed from the membrane. A prolonged centrifugation at >12,000 × *g* can aid in removal of wash buffer.
	Inefficient membrane wash.	If the collection tube is overfilled during the wash step, some of the wash buffer may remain in the bottom of the purification column. To avoid this, always discard the flow-through after centrifugation.

Observation	Possible cause	Recommended action
Downstream applications are unsuccessful *(continued)*	Eluate contaminated with agarose.	Ensure the gel slice is properly solubilized. See "Dissolve gel" on page 7.
		Verify that a 1:1 volume of Binding Buffer was added to a precisely weighted gel slice. Large amounts of agarose or agarose gel percentages >2% may take more time to dissolve. In some cases, adding a larger volume of Binding Buffer and vortexing the gel solution more frequently can facilitate solubilization.
	Eluate contaminated with excess salt.	Ensure that the wash in step 4 of "Purify DNA" on page 8 using centrifuge or step 6 of "Purify DNA" on page 9 using vacuum manifold, is effective. Incubate the purification column with the Wash Buffer for several minutes before proceeding to centrifugation.

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Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

C Documentation and support

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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