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GeneJET™ PCR Purification Kit

USER GUIDE

Catalog Numbers K0701, K0702

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

ThermoFisher SCIENTIFIC

Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0012662 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.

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™ PCR Purification Kit is designed for rapid and efficient purification of DNA from PCR and other enzymatic reaction mixtures. The kit uses a proprietary silica-based membrane technology in the form of a convenient spin column, eliminating the need for tedious resin manipulations or toxic phenol-chloroform extractions.

The GeneJET™ PCR Purification Kit effectively removes primers, dNTPs, unincorporated labeled nucleotides, enzymes and salts from PCR and other reaction mixtures. The kit can be used for purification of DNA fragments from 25 bp to 20 kb. The recovery rates are 90–100% in a 100 bp–10 kb DNA fragment size range (see Figure 1). Each GeneJET™ purification column has a total binding capacity of up to 25 µg of DNA and the entire procedure takes 5 minutes. The purified DNA can be used in common downstream applications such as sequencing, restriction digestion, labeling, ligation, cloning, in vitro transcription, blotting or in situ hybridization.

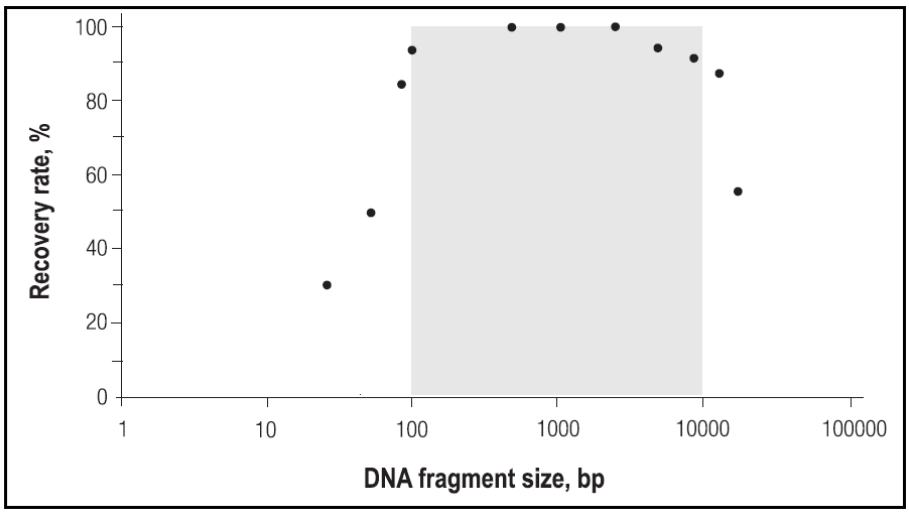

Figure 1 Recovery dependence on DNA fragment size

Technology overview

A reaction mixture containing DNA is combined with the binding buffer and added to a purification column. A chaotropic agent in the binding buffer 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™ PCR Purification Kit

Component	Cat. No. K0701 (50 reactions)	Cat. No. K0702 (250 reactions)	Storage
Binding Buffer	12 mL	60 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	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.
- Perform all purification steps at room temperature (15–25°C).

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. K0701 (50 reactions)	Cat. No. K0702 (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

Purify DNA using centrifuge

1. Add a 1:1 volume of Binding Buffer to complete PCR reaction mixture. Mix thoroughly.

   Note: For example, for 100 µL of PCR reaction mixture, add 100 µL of Binding Buffer.

2. If the color of the solution is orange or violet, add 10 µL of 3 M sodium acetate, pH 5.2 solution, then mix.

   Note: The color of the mix will become yellow. A yellow color indicates an optimal pH for DNA binding.

3. *(Optional)* If the DNA fragment is ≤500 bp, add a 1:2 volume of 100% isopropanol. Mix thoroughly.

   Note: For example, 100 µL of isopropanol should be added to the 100 µL PCR reaction mixture combined with 100 µL of Binding Buffer.

   Note: If the PCR reaction mixture contains primer-dimers, purification without isopropanol is recommended. However, the yield of the target DNA fragment will be lower.

4. Transfer up to 800 µL of the solution from step 1 (or optional step 2) to the purification column. Centrifuge at >12,000 × *g* for 30–60 seconds. Discard the flow-through.

   Note: If the total volume exceeds 800 µL, the solution can be added to the column in stages. After the addition of 800 µL of solution, centrifuge the column at >12,000 × *g* for 30–60 seconds, then discard flow-through. Repeat until the entire solution has been added to the column membrane.

5. Add 700 µL of Wash Buffer supplemented with ethanol (see “Before first use of the kit” on page 6) to the purification column.

6. Centrifuge at >12,000 × *g* for 30–60 seconds. Discard the flow-through, then place the purification column back into the collection tube.

7. Centrifuge the empty purification column at >12,000 × *g* for1 minute to completely remove any residual wash buffer.

   IMPORTANT! This step is essential as the presence of residual ethanol in the DNA sample may inhibit subsequent reactions.

8. Transfer the purification column to a clean 1.5 mL microcentrifuge tube.

9. 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.

Purify DNA using vacuum manifolds

1. Perform DNA binding stage according to step 1– step 3 for "Purify DNA using centrifuge" on page 7.
2. Follow supplier instructions to prepare the vacuum manifold, then place the purification column(s) onto the manifold.

Note: Close the bag with purification columns tightly after each use.

3. Transfer up to 800 µL of the solution to the purification column.

Note: If the total volume exceeds 800 µL, the solution can be added to the column in stages. After the addition of 800 µL of solution, centrifuge the column at >12,000 × *g* for 30–60 seconds, then discard flow-through. Repeat until the entire solution has been added to the column membrane.

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. Add 700 µL of Wash Buffer supplemented with ethanol ("Before first use of the kit" on page 6) to the purification column.
6. Apply the vacuum to draw the solution through the column. Switch off the vacuum after the solution has passed through the column.
7. Place the purification column back into the collection tube.
8. Centrifuge the empty purification column at >12,000 × *g* for 1 minute to completely remove any residual wash buffer.

IMPORTANT! This step is essential as the presence of residual ethanol in the DNA sample may inhibit subsequent reactions.

9. Transfer the purification column to 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	Inefficient DNA binding.	Verify that a 1:1 volume of Binding Buffer is added to the reaction mixture.
		Ensure the solutions are mixed well.
		Check the color of the solution. 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 the recommended volume of ethanol has been added to the Wash Buffer (concentrated) prior first use (see "Before first use of the kit" on page 6).
	Inefficient DNA elution.	Add 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 (e.g., >15 µg).
		When centrifuging the empty purification column, ensure all residual wash buffer is removed from the membrane. Longer centrifugation time (extra minute) can aid in removal of wash buffer.
	PCR reaction mixture does not contain DNA.	Check for the presence and yield of the PCR product by running an aliquot of the reaction on an agarose gel.
Downstream reactions are unsuccessful	Presence of residual ethanol.	On step 7 of "Purify DNA using centrifuge" on page 7 or step 8 of "Purify DNA using vacuum manifolds" on page 8, ensure all residual wash buffer is removed from the membrane. Longer centrifugation time can aid in removal of wash buffer.
	Inefficient membrane wash.	Ensure that the collection tube is not overfilled during the wash step and that any of the wash buffer has remained at the bottom of the purification column. Always discard the flow-through after centrifugation.
	Eluate contains excess salt.	Ensure that the wash on step 5 of "Purify DNA using centrifuge" on page 7 or step 5 of "Purify DNA using vacuum manifolds" on page 8 is effective. Incubate the purification column with the Wash Buffer for several minutes before proceeding to centrifugation.
DNA does not remain in an agarose gel well	DNA does not remain in an agarose gel well.	In step 7 of "Purify DNA using centrifuge" on page 7 or step 8 of "Purify DNA using vacuum manifolds" on page 8, ensure all residual wash buffer is removed from the membrane. Longer centrifugation time can aid in removal of wash buffer.

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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

Customer and technical support

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- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.