Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific GeneJET Gel Extraction Kit

#K0691, #K0692

www.thermoscientific.com/onebio

#__
Lot __ Expiry date _

CERTIFICATE OF ANALYSIS

The kit was tested in the extraction of 100 bp DNA fragment from a 2% agarose gel and a 5.5 kb DNA fragments from 1% agarose gel according to the protocol described in the manual. The quality of the extracted DNA was evaluated spectrophotometrically, by agarose gel electrophoresis, digestion with Thermo Scientific FastDigest restriction enzymes and automated fluorescent sequencing.

Quality authorized by:  Jurgita Zilinskiene

Rev.10

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COMPONENTS OF THE KIT

GeneJET Gel Extraction Kit	**50 preps** #K0691	**250 preps** #K0692
Binding Buffer	30 mL	150 mL
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

STORAGE AND STABILITY

The Thermo Scientific GeneJET Gel Extraction Kit should be stored at room temperature (15-25°C). For columns we recommend 4°C storage for periods greater than 1 year. Any precipitate that forms in the buffers during storage can be redissolved by incubating briefly at 37°C, then cooling to room temperature before use.

Note. Close the bag with GeneJET Purification Columns tightly after each use!

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 utilizes 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* Fig. 1). Each GeneJET 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 just 15 min and the isolated DNA is ready to use in all common downstream applications including ligation, restriction digestion, PCR, sequencing and labeling.

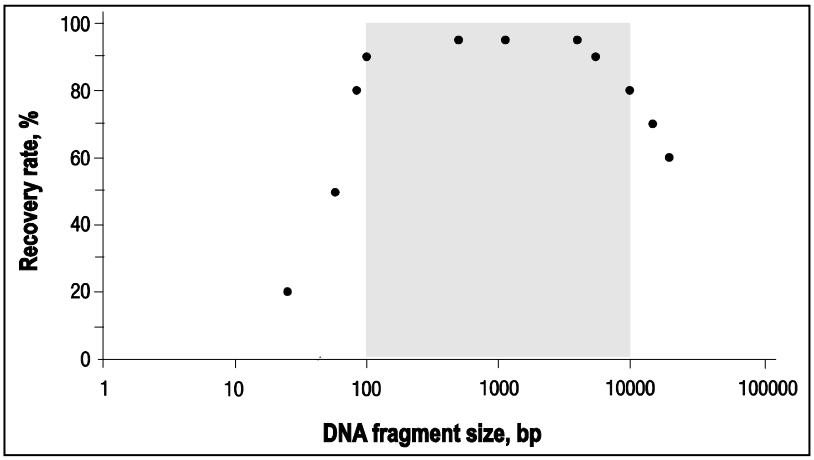

Fig. 1. Recovery dependence on DNA fragment size

PRINCIPLE

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.

IMPORTANT NOTES

- Prior to the initial use of the kit, dilute the **Wash Buffer** (concentrated) with ethanol (96-100%):

	50 preps #K0691	**250 preps** #K0692
Wash Buffer (concentrated)	9 mL	45 mL
Ethanol	45 mL	225 mL
Total Volume	54 mL	270 mL

  After the ethanol has been added, mark the check box on the bottle to indicate the completed step.
- Examine the **Binding Buffer** for precipitates before each use. Re-dissolve any precipitate by warming the solution to 37°C and cooling to 25°C.
- Wear gloves when handling the **Binding Buffer** as this solution contains irritants (*see* p.7 for SAFETY INFORMATION).
- Do not reuse electrophoresis buffer when extracted DNA fragment will be used directly for sequencing.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Ethanol 96-100%.
- Isopropanol.
- 3 M sodium acetate, pH 5.2 (may be necessary).
- Microcentrifuge.
- 1.5 or 2 mL microcentrifuge tubes.
- Heating block or water bath.

PURIFICATION PROTOCOL

Note

- Read IMPORTANT NOTES on p. 3 before starting.
- All purification steps should be carried out at **room temperature**.
- All centrifugations should be carried out in a table-top microcentrifuge at **>12000 × g** (10 000-14 000 rpm, depending on the rotor type).

Step	Procedure
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. Place the gel slice into a pre-weighed 1.5 mL tube and weigh. Record the weight of the gel slice. **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	Add **1:1 volume** of **Binding Buffer** to the gel slice (volume: weight) (e.g., add 100 µL of Binding Buffer for every 100 mg of agarose gel). **Note.** For gels with an agarose content greater than 2%, add 2:1 volumes of Binding Buffer to the gel slice.
3	Incubate the gel mixture at **50-60°C** for **10 min** or until the gel slice is completely dissolved. Mix the tube by inversion every few minutes to facilitate the melting process. Ensure that the gel is completely dissolved. Vortex the gel mixture briefly before loading on the column. 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 and mix. The color of the mix will become yellow.
4 for ≤500 bp and >10 kb DNA fragments	*Optional:* use this step only when DNA fragment is ≤500 bp or >10 kb long. • If the DNA fragment is ≤500 bp, add 1 gel volume of 100% isopropanol to the solubilized gel solution (e.g. 100 µL of isopropanol should be added to 100 mg gel slice solubilized in 100 µL of Binding Buffer). Mix thoroughly. • If the DNA fragment is >10 kb, add 1 gel volume of water to the solubilized gel solution (e.g. 100 µL of water should be added to 100 mg gel slice solubilized in 100 µL of Binding Buffer). Mix thoroughly.
5	Transfer up to 800 µL of the solubilized gel solution (from step 3 or 4) to the GeneJET purification column. Centrifuge for 1 min. Discard the flow-through and 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 the column for 30-60 s and 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. • Close the bag with GeneJET Purification Columns tightly after each use!

Step	Procedure
6	*Optional:* use this additional binding step only if the purified DNA will be used for sequencing. Add **100 µL** of **Binding Buffer** to the GeneJET purification column. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
7	Add **700 µL** of **Wash Buffer** (diluted with ethanol as described on p. 3) to the GeneJET purification column. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube.
8	Centrifuge the empty GeneJET purification column for an additional 1 min to completely remove residual wash buffer. **Note.** 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 GeneJET purification column into a clean 1.5 mL microcentrifuge tube (not included). Add **50 µL** of **Elution Buffer** to the center of the purification column membrane. Centrifuge for 1 min. **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 min at room temperature before centrifugation.
10	Discard the GeneJET purification column and store the purified DNA at -20°C.

TROUBLESHOOTING

Problem	Possible Cause and Solution
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 (e.g. for every 100 mg of agarose gel, add 100 µL of Binding Buffer). Ensure that the gel slice is completely dissolved before applying to the GeneJET purification column. A large amount of agarose or a gel slice with an agarose percentage greater than 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* p. 3). **Inefficient DNA elution** Add the Elution Buffer directly to the center of the membrane and not to the side of the GeneJET 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). In step 8, 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 8, 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.

Problem	Possible Cause and Solution
Downstream applications are unsuccessful	**Presence of residual ethanol** In step 8, ensure all residual wash buffer is removed from the membrane. A prolonged centrifugation time 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 GeneJET purification column. To avoid this, always discard the flow-through after centrifugation. **Eluate contaminated with agarose** Ensure the gel slice is properly solubilized during steps 1-4. 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 greater than 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 7 is effecitve. Incubate the GeneJET purification column with the Wash Buffer for several minutes before proceeding to centrifugation.

References

1. Vogelstein, B. and Gillespie, D., Preparative and analytical purification of DNA from agarose, Proc. Natl. Acad. Sci. USA, 76, 615-619, 1979.
2. Marko, M.A., Chipperfield, R. and Birnboim, H.C., A procedure for the large-scale isolation of highly purified plasmid DNA using alkaline extraction and binding to glass powder, Anal. Biochem., 121, 382-387, 1982.
3. Boom, R., Sol, C.J.A., et al., Rapid and simple method for purification of nucleic acids, J. Clin. Microbiol., Mar, 495-503, 1990.

SAFETY INFORMATION

Binding Buffer
Hazard-determining component of labeling: **guanidinium thiocyanate**

Xn Harmful

Risk phrases

R20/21/22 Harmful by inhalation, in contact with skin and if swallowed.
R32 Contact with acids liberates very toxic gas.
R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Safety phrases

S9 Keep container in a well-ventilated place.
S23 Do not breathe gas/fumes/vapour/spray.
S36/37 Wear suitable protective clothing and gloves.
S60 This material and its container must be disposed of as hazardous waste.
S61 Avoid release to the environment. Refer to special instructions/safety data sheets.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.