GeneJET™ PCR Purification Kit

#K0701, #K0702

Rev.4 71717171

CERTIFICATE OF ANALYSIS

GeneJET™ PCR Purification Kit

#K0702
Lot ___

QUALITY CONTROL

The kit was tested in the purification of 100 bp and 5.5 kb PCR products according to the protocol described in the manual. The quality of the purified DNA was evaluated spectrophotometrically, by agarose gel electrophoresis, digestion with FastDigest® restriction enzymes and automated fluorescent sequencing.

Quality authorized by: Jurgita Zilinskiene

CONTENTS page

COMPONENTS OF THE KIT

GeneJET™ PCR Purification Kit	**50 preps** #K0701	**250 preps** #K0702
Binding Buffer	12 ml	60 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

GeneJET™ PCR Purification Kit can be stored for up to 12 months at room temperature (15-25°C) or at 4°C for storage periods longer than 12 months. Any precipitate in the buffers can be re-dissolved by incubating at 37°C before use.

DESCRIPTION

The GeneJET™ PCR Purification Kit is designed for rapid and efficient purification of DNA from PCR and other enzymatic reaction mixtures.
The kit utilizes 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* Fig. 1). Each GeneJET™ purification column has a total binding capacity of up to 25 µg of DNA and the entire procedure takes just 5 min. 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.

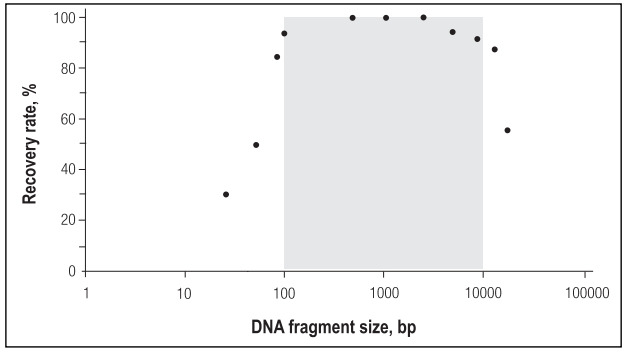

Fig. 1. Recovery dependence on DNA fragment size

PRINCIPLE

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.

IMPORTANT NOTES

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

	50 preps #K0701	**250 preps** #K0702
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.8 for SAFETY INFORMATION).

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 (may be necessary).

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 x g** (10 000-14 000 rpm, depending on the rotor type).

Step	**Procedure**
1	Add a **1:1 volume** of **Binding Buffer** to completed PCR mixture (e.g. for every 100 µl of reaction mixture, add 100 µl of Binding Buffer). Mix thoroughly. 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.
2 for DNA ≤500 bp	*Optional:* if the DNA fragment is ≤500 bp, add a 1:2 volume of 100% isopropanol (e.g., 100 µl of isopropanol should be added to 100 µl of PCR mixture combined with 100 µl of Binding Buffer). Mix thoroughly. **Note.** If PCR mixture contains primer-dimers, purification without isopropanol is recommended. However, the yield of the target DNA fragment will be lower.
3	Transfer up to 800 µl of the solution from step 1 (or optional step 2) to the GeneJET™ purification column. Centrifuge for 30-60 s. 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 for 30-60 s and discard flow-through. Repeat until the entire solution has been added to the column membrane.
4	Add **700 µl** of **Wash Buffer** (diluted with the ethanol as described on p. 3) to the GeneJET™ purification column. Centrifuge for 30-60 s. Discard the flow-through and place the purification column back into the collection tube.
5	Centrifuge the empty GeneJET™ purification column for an additional 1 min to completely remove any residual wash buffer. **Note.** This step is essential as the presence of residual ethanol in the DNA sample may inhibit subsequent reactions.
6	Transfer the GeneJET™ purification column to a clean 1.5 ml microcentrifuge tube (not included). Add **50 µl** of **Elution Buffer** to the center of the GeneJET™ purification column membrane and 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.
7	Discard the GeneJET™ purification column and store the purified DNA at -20°C.

TROUBLESHOOTING

Problem	Possible Cause and Solution
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* p. 3). **Inefficient DNA elution** Add 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 5, 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** In step 5, 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 in the bottom of the GeneJET™ purification column. Always discard the flow-through after centrifugation. **Eluate contains excess salt** Ensure that the wash step 4 of the protocol is effective. Incubate the GeneJET™ purification column with the Wash Buffer for several minutes before proceeding to centrifugation.
DNA does not remain in an agarose gel well	In step 5, ensure all residual wash buffer is removed from the membrane. Longer centrifugation time can aid in removal of wash buffer.

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.

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.

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.fermentas.com for Material Safety Data Sheet of the product.