Thermo
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PRODUCT INFORMATION

Thermo Scientific GeneJET Gel Extraction and DNA Cleanup Micro Kit

#K0831, #K0832

www.thermoscientific.com/onebio

#__
Lot __
Expiry Date _

CERTIFICATE OF ANALYSIS

The Thermo Scientific GeneJET Gel Extraction and DNA Cleanup Micro Kit is qualified by isolating 3 kb DNA fragment from 200 µL reaction mixture and extracting 3 kb DNA from 1% agarose gel according the protocols outlined in the manual. The quality of the purified and extracted DNA is evaluated spectrophotometrically and by agarose gel electrophoresis.

Quality authorized by:  Jurgita Zilinskiene

Rev.1. f

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COMPONENTS OF THE KIT

GeneJET Gel Extraction and DNA Cleanup Micro Kit	50 preps #K0831	250 preps #K0832
Binding Buffer	5.5 mL	28 mL
Extraction Buffer	11 mL	55 mL
Prewash Buffer (concentrated)	10 mL	44 mL
Wash Buffer (concentrated)	2 × 7 mL	2 × 40 mL
Elution Buffer (10 mM Tris-HCl, pH 8.5)	1.5 mL	4 × 1.5 mL
GeneJET DNA Purification Micro Column & Collection Tube	50	250

STORAGE AND STABILITY

GeneJET™ Gel Extraction and DNA Cleanup Micro Kit can be stored for up to 12 months at room temperature (15-25°C).

DESCRIPTION

The GeneJET Gel Extraction and DNA Cleanup Micro Kit is developed as 3 in 1 kit designed for rapid and efficient purification of DNA from PCR, enzymatic reaction mixtures, and DNA extraction from standard or low-melting point agarose gels run in either Tris acetate (TAE) or Tris borate (TBE) buffer.

The kit combines the convenience of spin column technology with the selective binding properties of a silica membrane, eliminating the need for tedious resin manipulations or toxic phenol-chloroform extractions.

The GeneJET Gel Extraction and DNA Cleanup Micro Kit effectively removes primers, primer dimers, dNTPs, unincorporated labeled nucleotides, enzymes and salts from PCR and other reaction mixtures. The kit can be used for purification of DNA fragments from 100 bp to 20 kb**.** The recovery rates are 90-100% for 100 bp – 4 kb DNA fragment size range. Each GeneJET DNA Purification Micro Column has a total binding capacity of up to10 µg of DNA, and the entire procedure takes approximately 3.5 minutes for DNA cleanup from enzymatic reaction or 15 minutes for DNA extraction from gel.

The purified DNA can be used in common downstream applications such as sequencing, restriction enzyme digestion, PCR, qPCR, labeling, ligation, cloning, *in vitro* transcription, blotting or *in situ* hybridization.

PRINCIPLE

The GeneJET Gel Extraction and DNA Cleanup Micro Kit is based on the ability of DNA to bind to silica membranes in the presence of chaotropic salts. DNA adsorbs to the silica membrane while contaminants pass through the column. Alternatively, after electrophoresis to separate the DNA fragments, the band(s) of interest is excised from an agarose gel and dissolved in Extraction Buffer, then mixed with ethanol and loaded on GeneJET DNA Purification Micro Column. Impurities are subsequently removed from the silica membrane by the addition of the Prewash Buffer and Wash Buffer, and the pure DNA is effectively eluted with Elution Buffer. The purified DNA is used for a wide variety of downstream applications.

IMPORTANT NOTES

- Add the indicated volume of ethanol (96-100%) to the **Prewash Buffer** (concentrated) and **Wash Buffer** (concentrated) prior to the first use:

	#K0831 50 preps		**#K0832 250 preps**	
	Prewash Buffer (1 bottle)	**Wash Buffer (2 bottles)**	**Prewash Buffer (1 bottle)**	**Wash Buffer (2 bottles)**
Concentrated solution	10 mL	7 mL	44 mL	40 mL
Ethanol (96-100%)	**2.5 mL**	**35 mL**	**11 mL**	**200 mL**
Total volume:	12.5 mL	42 mL	55 mL	240 mL

- After the ethanol has been added, mark the check box on the bottle to indicate the completed step.
- Check all solutions in the kit for any salt precipitation before each use. Re-dissolve any precipitate by warming the solution to 37°C, and then equilibrate to room temperature (15-25°C).
- Wear gloves when handling the **Binding Buffer and Extraction Buffer** as these solutions contain irritants and are harmful if they come into contact with skin, are inhaled or swallowed (*see* p. 8 for SAFETY INFORMATION)
- Do not re-use electrophoresis buffer when extracted DNA fragment will be used directly for sequencing.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Water, nuclease-free or TE buffer
- Ethanol (96-100%)
- Pipettes
- Microcentrifuge
- 1.5 or 2 mL microcentrifuge tubes
- Heating block or water bath (necessary for DNA extraction from gel)
- Disposable gloves

PURIFICATION PROTOCOL

Notes

- Read IMPORTANT NOTES on p. 3 before starting.
- All purification steps should be carried out at **room temperature (15-25°C)**.
- If DNA fragment is ≥ 10 kb all centrifugations should be carried out no less than 2 minutes.

A. General DNA cleanup from enzymatic reactions protocol.

Step	Procedure
1	Adjust the volume of the reaction mixture to 200 µL with Water, nuclease-free or TE buffer (not included).
2	Add 100 µL of **Binding Buffer**. Mix thoroughly by pipetting.
3	Add 300 µL of ethanol (96-100%) and mix by pipetting.
4	Transfer the mixture to the **GeneJET DNA Purification Micro Column** preassembled with a collection tube. Centrifuge the column for 30-60 seconds at 14,000 × g. Discard the flow-through. Place the GeneJET DNA Purification Micro Column back into the collection tube. **Note.** If DNA fragment is ≥ 10 kb centrifuge the column for 2 minutes at 14,000 × g.
5	Add 700 µL of **Wash Buffer** (supplemented with ethanol, see p. 3) to the GeneJET DNA Purification Micro Column and centrifuge for 30-60 seconds at 14,000 × g. Discard the flow-through and place the purification column back into the collection tube. **Note.** If DNA fragment is ≥ 10 kb centrifuge the column for 2 minutes at 14,000 × g.
6	Repeat step 5.
7	Centrifuge the empty GeneJET DNA Purification Micro Column for an additional 1 minute at 14,000 × g 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.
8	Transfer the GeneJET DNA Purification Micro Column into a clean 1.5 mL microcentrifuge tube (not included).
9	Add 10 µL of **Elution Buffer** to the center of the GeneJET DNA Purification Micro Column membrane. Centrifuge for 1 minute at 14,000 × g to elute DNA. **Note**. • If DNA fragment is ≥ 10 kb the elution volume should be increased to 15-20 µL to get optimal DNA yield. • Lower volume of Elution Buffer for DNA Micro Kit can be used (6-10 µL) in order to concentrate eluted DNA. Please notice that < 10 µL elution volume slightly decreases DNA yield. • Double the elution volume or perform two elution cycles when purifying larger amounts of DNA (for example > 5 µg).
10	Discard the purification column and store the purified DNA at -20°C.

B. PCR cleanup, dimers removal protocol.

Step	Procedure
1	Adjust the volume of the reaction mixture to 200 µL with Water, nuclease-free or TE buffer (not included).
2	Add 100 µL of **Binding Buffer**. Mix thoroughly by pipetting.
3	Add 300 µL of ethanol (96-100%) and mix by pipetting.
4	Transfer the mixture to the **GeneJET DNA Purification Micro Column** preassembled with a collection tube. Centrifuge the column for 30-60 seconds at 14,000 × g. Discard the flow-through. Place the GeneJET DNA Purification Micro Column back into the collection tube. **Note.** If DNA fragment is ≥ 10 kb centrifuge the column for 2 minutes at 14,000 × g.
5	Add 200 µL of **Prewash Buffer** (supplemented with ethanol, see p. 3) to the GeneJET DNA Purification Micro Column and centrifuge for 30-60 seconds at 14,000 × g. Discard the flow-through and place the purification column back into the collection tube. **Note.** If DNA fragment is ≥ 10 kb centrifuge the column for 1-2 minutes at 14,000 × g.
6	Add 700 µL of **Wash Buffer** (supplemented with ethanol, see p. 3) to the GeneJET DNA Purification Micro Column and centrifuge for 30-60 seconds at 14,000 × g. Discard the flow-through and place the purification column back into the collection tube. **Note.** If DNA fragment is ≥ 10 kb centrifuge the column for 1-2 minutes at 14,000 × g.
7	Repeat step 6.
8	Centrifuge the empty GeneJET DNA Purification Micro Column for an additional 1 minute at 14,000 × g 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 DNA Purification Micro Column into a clean 1.5 mL microcentrifuge tube (not included).
10	Add 10 µL of **Elution Buffer** to the center of the GeneJET DNA Purification Micro Column membrane. Centrifuge for 1 minute at 14,000 × g to elute DNA. **Note**. • If DNA fragment is ≥ 10 kb the elution volume should be increased between 15-20 µL to get optimal DNA yield. Elution volume less than 10 µL is not recommended. • Lower volume of Elution Buffer for DNA Micro Kit can be used (6-10 µL) in order to concentrate eluted DNA. Please notice that < 10 µL elution volume slightly decreases DNA yield. • Double the elution volume or perform two elution cycles when purifying larger amounts of DNA (for example > 5 µg).
11	Discard the purification column and store the purified DNA at -20°C.

C. DNA extraction from gel protocol.

Step	Procedure
1	Excise up to 200 mg 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 1.5 mL tube. **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 200 µL of **Extraction Buffer**. Mix thoroughly by pippeting.
3	Incubate the gel mixture at **50-58°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. Ensure that the gel is completely dissolved. **Note.** For > 1% agarose gels prolong the incubation time to 15 min.
4	Add 200 µL of ethanol (96-100%) and mix by pipetting.
5	Transfer the mixture to the **GeneJET DNA Purification Micro Column** preassembled with a collection tube. Centrifuge the column for 30-60 seconds at 14,000 × g. Discard the flow-through. Place the GeneJET DNA Purification Micro Column back into the collection tube. **Note.** If DNA fragment is ≥ 10 kb centrifuge the column for 2 minutes at 14,000 × g.
6	Add 200 µL of **Prewash Buffer** (supplemented with ethanol, see p. 3) to the GeneJET DNA Purification Micro Column and centrifuge for 30-60 seconds at 14,000 × g. Discard the flow-through and place the purification column back into the collection tube. **Note.** If DNA fragment is ≥ 10 kb centrifuge the column for 1-2 minutes at 14,000 × g.
7	Add 700 µL of **Wash Buffer** (supplemented with ethanol, see p. 3) to the GeneJET DNA Purification Micro Column and centrifuge for 30-60 seconds at 14,000 × g. Discard the flow-through and place the purification column back into the collection tube. **Note.** If DNA fragment is ≥ 10 kb centrifuge the column for 1-2 minutes at 14,000 × g.
8	Repeat step 7.
9	Centrifuge the empty GeneJET DNA Purification Micro Column for an additional 1 minute at 14,000 × g 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.
10	Transfer the GeneJET DNA Purification Micro Column into a clean 1.5 mL microcentrifuge tube (not included).
11	Add 10 µL of **Elution Buffer** to the GeneJET DNA Purification Micro Column. Centrifuge for 1 minute at 14,000 × g to elute DNA. **Note**. • If DNA fragment is ≥ 10 kb the elution volume should be increased between 15-20 µL to get optimal DNA yield. Elution volume less than 10 µL is not recommended. • Lower volume of Elution Buffer for DNA Micro Kit can be used (6-10 µL) in order to concentrate eluted DNA. Please notice that < 10 µL elution volume slightly decreases DNA yield.
12	Discard the purification column and store the purified DNA at -20°C.

TROUBLESHOOTING

Problem	Possible Cause and Solution
Low DNA yield	**Inefficient DNA binding.** Verify that the **Binding Buffer** was added to the reaction mixture. Ensure the solutions are mixed well. For DNA extraction from gel - ensure under UV light that all band of interest was cut out and DNA band was sharp, not smeared or curved. **Ethanol was not added to the DNA sample.** Ensure ethanol was added to the DNA sample and **Binding Buffer** mixture before applying the sample to the GeneJET DNA Purification Micro Column**.** Ensure that the recommended volume of ethanol has been added to the reaction mixture or dissolved DNA gel sample. **Ethanol was not mixed with the sample.** After the addition of ethanol to mixture, mix the sample by pipetting. **Inefficient membrane wash.** Ensure that the recommended volume of ethanol has been added to the **Prewash Buffer** (concentrated) and **Wash Buffer** (concentrated) prior the first use (*see* p. 3). **Inefficient DNA elution.** Double the volume of **Elution Buffer** or perform two elution cycles when purifying larger amounts of DNA (> 5 µg). **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 the empty GeneJET DNA Purification Micro Column centrifugation step, 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 no wash buffer has remained in the bottom of the GeneJET purification column after centrifugation. Always discard the flow-through after centrifugation.
DNA does not remain in an agarose gel well	In the empty GeneJET DNA Purification Micro Column centrifugation step, ensure all residual wash buffer is removed from the membrane. Longer centrifugation time can aid in removal of wash buffer.

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

Xn Harmful

Risk phrases

R20/21/22	Harmful by inhalation, 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.

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

Xn Harmful

Risk phrases:

22	Harmful if swallowed.
38	Irritating to skin.
41	Risk of serious damage to eyes.
52/53	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Safety phrases

23	Do not breathe gas/fumes/vapour/spray.
26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
36/37/39	Wear suitable protective clothing, gloves and eye/face protection.
60	This material and its container must be disposed of as hazardous waste.
61	Avoid release to the environment. Refer to special instructions/safety data sheets.

Patent pending

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.