Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific GeneJET NGS Cleanup Kit

#K0851, #K0852

www.thermoscientific.com/onebio

#K0851, K0852
Lot __
Expiry Date _

CERTIFICATE OF ANALYSIS

Thermo Scientific™ GeneJET™ NGS Cleanup Kit has been tested by isolating of 300 bp DNA fragment from reaction mixture according the protocol outlined in the manual. The quality of the purified DNA is evaluated spectrophotometrically and by agarose gel electrophoresis.

Quality authorized by:  Jurgita Zilinskiene

Rev.1. 69

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COMPONENTS OF THE KIT

GeneJET NGS Cleanup Kit	#K0851 50 preps	#K0852 250 preps
Binding Buffer for GeneJET NGS Cleanup Kit	35 mL	165 mL
Prewash Buffer (conc.) for GeneJET NGS Cleanup Kit	20 mL	2 × 44 mL
Wash Buffer (conc.) for GeneJET NGS Cleanup Kit	2 × 7 mL	2 × 40 mL
Elution Buffer (10 mM Tris-HCl, pH 8.5)	15 mL	2 × 18 mL
DNA Purification Micro Column & Collection Tube	50	250
Collection Tube	50	250

STORAGE AND STABILITY

The GeneJET NGS Cleanup Kit should be stored at room temperature (15 – 25 °C) until kit expiration date.

Note: Close the DNA Purification Micro Column & Collection Tube bag tightly after each use!

DESCRIPTION

The GeneJET NGS Cleanup Kit is designed for efficient cleanup of DNA fragments for next generation sequencing (NGS) applications. The kit is compatible with a variety of enzymatic reaction mixtures, including PCR, ligation or DNA end repair mixes, and efficiently removes any interfering reaction components such as sequencing adapters, primer dimers, nucleotides, enzymes and salts.

The GeneJET NGS Cleanup Kit provides protocols optimized specially for fast DNA fragments cleanup or adapter removal procedures. The cleanup protocol results in higher than 70% recovery of DNA fragments larger than 150 bp and more than 97% removal of adapters. Adapter removal protocol results in more than 70% recovery of DNA fragments larger than 200 bp and more than 99% removal of adapters. The entire procedure takes 5 minutes for DNA fragment library cleanup from enzymatic reaction mixtures and 7 minutes for adapter removal protocol. Spin columns technology in combination with unique binding buffer allows to get the reproducible yields of DNA fragments in the wide starting material range (5 ng – 1 µg of DNA fragments).

The GeneJET NGS Cleanup Kit is compatible with Illumina, Life Technologies library preparation workflows.

PRINCIPLE

The Kit is based on the ability of DNA to bind to silica membranes in the presence of chaotropic salts. DNA fragments larger than 120 bp adsorb to the silica membrane while smaller fragments and contaminants pass through the column. Adapters are subsequently removed from the silica membrane by the addition of Prewash Buffer, and the pure DNA fragment library is effectively eluted with Elution Buffer.

IMPORTANT NOTES

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

	#K0851 50 preps		**#K0852 250 preps**	
	Prewash Buffer (1 bottle)	**Wash Buffer (2 bottles)**	**Prewash Buffer (1 bottle)**	**Wash Buffer (2 bottles)**
Concentrated solution	20 mL	7 mL	44 mL	40 mL
Ethanol (96-100%)	**5 mL**	**35 mL**	**11 mL**	**200 mL**
Total volume:	25 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** as this solution contain irritants that are harmful if they come into contact with skin, are inhaled or swallowed (*see* page 9 for SAFETY INFORMATION)

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Ethanol (96 - 100%)
- Pipettes
- Vortex
- Microcentrifuge
- 1.5 or 2 mL microcentrifuge tubes or LoBind™ Tubes 1.5 mL (Cat. #022431021, Eppendorf)
- Disposable gloves

PURIFICATION PROTOCOLS

Notes

- Read IMPORTANT NOTES on page 5 before starting.
- All purification steps should be carried out at **room temperature (15 – 25 °C)**.

Protocol A. DNA fragments cleanup protocol.

Step	Procedure
1	Add 5 volumes of **Binding Buffer** to 1 volume of DNA fragments sample**.** For example, mix 250 µL Binding Buffer with 50 µL of sample. **Note. Use up to 100 µL of starting material volume.**
2	Add 1 DNA fragments volume of **ethanol** (96 - 100%) and mix by pipetting or vortexing. For example, if 50 µL of starting material is used, add 50 µL of ethanol.
3	Transfer the mixture to the **DNA Purification Micro Column** preassembled with a collection tube. Centrifuge the column for 30 seconds at 10,000 × g. Discard the flow-through. Place the DNA Purification Micro Column back into the collection tube.
4	Add 200 µL of **Prewash Buffer** (supplemented with ethanol, see page 5) to the DNA Purification Micro Column and centrifuge for 30 seconds at 10,000 × g. Discard the flow-through and place the purification column back into the collection tube.
5	Add 700 µL of **Wash Buffer (**supplemented with ethanol, see page 5) to the DNA Purification Micro Column and centrifuge for 30 seconds at 10,000 × g. Discard the flow-through and place the purification column back into the collection tube.
6	Repeat step **5**.
7	Centrifuge the empty DNA Purification Micro Column for an additional 2 minutes 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 DNA Purification Micro Column into a clean 1.5 mL microcentrifuge tube (not included).
9	Add 10 - 30 µL of **Elution Buffer** to the center of the DNA Purification Micro Column membrane. Centrifuge for 1 minute at 14,000 × g to elute **DNA fragment library.** **Note**. • Lower volume of Elution Buffer can be used (6-10 µL) in order to concentrate eluted DNA. Please note that < 10 µL elution volume slightly decreases DNA yield.
10	Discard the purification column and store the purified DNA at -20 °C.

Protocol B. Adapter removal protocol

Step	Procedure
1	Add 5 volumes of **Binding Buffer** to 1 volume of DNA fragments sample**.** For example, mix 250 µL Binding Buffer with 50 µL of sample. **Note. Use up to 100 µL of starting material volume.**
2	Add 1 DNA fragments volume of **ethanol** (96-100%) and mix by pipetting or vortexing. For example, if 50 µL of starting material is used, add 50 µL of ethanol.
3	Transfer the mixture to the **DNA Purification Micro Column** preassembled with a collection tube. Centrifuge the column for 30 seconds at 10,000 × g. Discard the flow-through. Place the DNA Purification Micro Column back into the collection tube.
4	Add 200 µL of **Prewash Buffer** (supplemented with ethanol, see page 5) to the DNA Purification Micro Column and centrifuge for 30 seconds at 10,000 × g. Discard the flow-through and place the Purification Micro column into a clean 1.5 mL microcentrifuge tube (not included).
5	Add 100 µL of **Elution Buffer** to the DNA Purification Micro Column and centrifuge for 30 seconds at 10,000 × g. Colect the flow-through into the 1.5 mL microcentrifuge tube. Place the DNA Purification Micro Column into a new collection tube (included). **Note.** Do not discard the DNA Purification Micro Column!
6	Add 100 µL of **Binding Buffer** to the collected flow-through. Mix by pipetting or vortexing**.**
8	Transfer the mixture to the same **DNA Purification Micro Column** added into a new collection tube. Centrifuge the column for 30 seconds at 10,000 × g. Discard the flow-through. Place the DNA Purification Micro Column back into the collection tube.
9	Add 200 µL of **Prewash Buffer** (supplemented with ethanol, see page 5) to the DNA Purification Micro Column and centrifuge for 30 seconds at 10,000 × g. Discard the flow-through and place the purification column back into the collection tube.
10	Add 700 µL of **Wash Buffer** (supplemented with ethanol, see page 5) to the DNA Purification Micro Column and centrifuge for 30 seconds at 10,000 × g. Discard the flow-through and place the purification column back into the collection tube.
11	Repeat step **10**.
12	Centrifuge the empty DNA Purification Micro Column for an additional 2 minutes 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.
13	Transfer the DNA Purification Micro Column into a clean 1.5 mL microcentrifuge tube (not included).
14	Add 10-30 µL of **Elution Buffer** to the center of the DNA Purification Micro Column membrane. Centrifuge for 1 minute at 14,000 × g to elute **DNA fragment library.** **Note**.Lower volume of Elution Buffer can be used (6-10 µL) in order to concentrate eluted DNA. Please note that < 10 µL elution volume decreases DNA yield.
15	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** and **ethanol** was added to the reaction mixture. Ensure the solutions are mixed well. **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 DNA Purification Micro Column**.** Ensure that the recommended volume of ethanol has been added to the reaction mixture. **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. 5). **Inefficient DNA elution.** Lower volume of Elution Buffer (< 10 µL) in order to concentrate eluted DNA slightly decreases DNA yield. **Reaction mixture does not contain DNA.** Check for the presence and yield of DNA by running an aliquot of the reaction on an agarose gel or analyzing with the Agilent 2100 Bioanalyzer.
Downstream reactions are unsuccessful	**Presence of residual ethanol.** In the empty 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 purification column after centrifugation. Always discard the flow-through after centrifugation.

SAFETY INFORMATION

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.

Note:
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.

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