GeneJET™ Plant Genomic DNA Purification Mini Kit

Catalog Numbers K0791, K0792

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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 Thermo Scientific™ GeneJET™ Plant Genomic DNA Purification Mini Kit is designed for rapid and efficient purification of high quality genomic DNA from wide variety of plant species and tissue types. The kit utilizes silica-based membrane technology in the form of a convenient spin column, eliminating the need for expensive resins, toxic phenol-chloroform extractions, or time-consuming alcohol precipitation.

The standard procedure takes less than 30 minutes following cell lysis and yields purified DNA of more than 30 kb in size. DNA yields vary between different species and tissues depending on genome size, ploidy, cell number, and age of tissue sample. The typical yield from the optimal source, such as young wheat leaves, is 30–32 µg from 100 mg of tissue. Isolated DNA can be used directly in PCR, qPCR, Southern blotting and enzymatic reactions.

Technology overview

Samples are lysed in supplied Lysis Buffers in the presence of RNase A. Proteins and polysaccharides are removed by Precipitation Solution. The lysate is then mixed with the Plant gDNA Binding Solution, ethanol and loaded on the purification column where the DNA binds to the silica membrane. Impurities are effectively removed by washing the column with the prepared wash buffers. Genomic DNA is then eluted under low ionic strength conditions with the Elution Buffer.

Contents and storage

IMPORTANT! Tightly seal the bag containing GeneJET™ Genomic DNA Purification Columns after each use.

Item	Cat. No. K0791 (50 reactions)	Cat. no. K0792 (250 reactions)	Storage
RNase A Solution	2 × 0.7 mL	6 × 1 mL	• Upon receipt, store the unopened vial at 15–25°C. • After use, store the vial at –20°C.
Lysis Buffer A	25 mL	120 mL	15–25°C
Lysis Buffer B	3 mL	15 mL	
Precipitation Solution	8 mL	40 mL	
Plant gDNA Binding Solution	24 mL	120 mL	
Wash Buffer 1 (concentrated)	10 mL	40 mL	
Wash Buffer 2 (concentrated)	10 mL	40 mL	
Elution Buffer (10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA)	10 mL	40 mL	
Collection Tubes (2 mL)	50	250	
GeneJET™ Genomic DNA Purification Columns and Collection Tubes	50	250	Store at 15–25°C for up to 6 months. For longer periods, store at 2–8°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
Equipment	
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
Vortex mixer, or equivalent	MLS
Thermo heating-bloks or waterbath (adjustable to 65°C and 90°C)	MLS
Centrifuge capable of ≥20,000 × *g* for 1.5 mL microcentrifuge tubes	MLS
Mortar and pestle or grinding mill	MLS
Tubes and other consumables	
Microcentrifuge tubes (1.5 mL) with screw caps	MLS
Disposable gloves	MLS
Ethanol, 96–100% (molecular biology grade)	MLS
Liquid nitrogen	MLS
Polyvinylpyrrolidone (PVP) (with lignified, polyphenol-rich plant tissues)	MLS
Dithiothreitol (DTT) (with rapeseeds)	MLS

Procedural guidelines

IMPORTANT! Wear gloves when handling the Plant gDNA Binding Solution and Wash Buffer I as these reagents contain irritants.

- To minimize DNA degradation, avoid repeated freezing and thawing of the samples and perform extractions from fresh material, material that has been immediately frozen and stored at -70°C, or lyophilized samples.
- Check the Lysis Solution B for salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use.
- Typically the purified genomic DNA has an A260/280 ratio between 1.7 and 1.9, however, when DNA concentration is lower than 20 ng/µL, deviations from the expected ratio are occasionally observed.

Before first use of the kit

1. Add the indicated volume of ethanol (96–100%) to Wash Buffer I (concentrated) and Wash Buffer II (concentrated) prior to first use:

–	Cat. no. K0791 (50 reactions)		Cat. no. K0792 (250 reactions)	
–	Wash Buffer I	Wash Buffer II	Wash Buffer I	Wash Buffer II
Concentrated wash solution	10 mL	10 mL	40 mL	40 mL
Ethanol (96–100%)	30 mL	30 mL	120 mL	120 mL
Total Volume	40 mL	40 mL	160 mL	160 mL

2. After ethanol has been added, mark the check box on the bottle's cap to indicate the completed step.

Purify DNA from liqified, polyphenol-rich plant tissues or rapeseeds

1. Pipette 350 µL of Lysis Buffer A into 1.5 mL microcentrifuge tube.
2. Weigh the plant tissue. Use up to 100 mg of fresh or frozen tissue or up to 20 mg of lyophilized tissue.

3. Grind the material by one of the following methods:
   - Place up to 100 mg of plant tissue into liquid nitrogen then grind thoroughly with a mortar and pestle.
   - Place up to 100 mg of tissue into a vial containing stainless steel beads. The vial and beads should be precooled with liquid nitrogen. The setup of the mechanical disruption depends on the tissue type.
4. Immediately transfer the tissue powder into a 1.5 mL microcentrifuge tube containing 350 µL of Lysis Buffer A. Vortex for 10–20 seconds to mix thoroughly.

   Note: Transfer the ground tissue to the Lysis Buffer as quickly as possible to avoid DNA degradation. All ground material must be thoroughly mixed with the Lysis Buffer. DNA degradation can occur in particles that are left to dry on the walls of the tube. Ground tissue can be used immediately in the DNA isolation protocol or stored at -70°C until use.
5. Add 50 µL of Lysis Buffer B and 20 µL RNase A.
6. *(Optional)* For tissues that are resistant to mechanical disruption, add glass sand to the microcentrifuge tube and vortex for 1 minute.
7. Incubate the sample at 65°C for 10 minutes. Vortex occasionally or use a shaking water bath, rocking platform or thermomixer.
8. Add 130 µL of Precipitation Solution then mix by inverting the tube 2–3 times. Incubate on ice for 5 minutes.
9. Centrifuge at ≥20,000 × *g* for 5 minutes. Collect the supernatant (usually 450–550 µL) then transfer to the clean microcentrifuge tube.
10. Add 400 µL of Plant gDNA Binding Solution and 400 µL of 96% ethanol then mix well.
11. Transfer half of the prepared mixture (600–700 µL) to the spin column. Centrifuge at 6,000 × *g* for 1 minute. Discard the flow-through solution then apply the remaining mixture onto the same column.
12. Centrifuge at 6,000 × *g* for 1 minute.

    IMPORTANT! Do not exceed specified relative centrifugal force.

    Note: Close the bag with GeneJET™ Genomic DNA Purification Columns tightly after each use.
13. Add 500 µL of Wash Buffer I to the column. Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through then place the column back into the collection tube.

    Note: Ensure ethanol has been added to Wash Buffer I.
14. Add 500 µL of Wash Buffer II to the column. Centrifuge at ≥20,000 × *g* for 3 mintues.

    Note: Ensure ethanol has been added to Wash Buffer II
15. Empty the collection tube. Place the purification column back into the tube then re-spin the column at ≥20,000 × *g* for 1 minute.
16. Discard the collection tube containing the flow-through solution then transfer the column to a sterile 1.5 mL microcentrifuge tube.
17. To elute genomic DNA, add 100 µL of Elution Buffer to the center of the column membrane. Incubate at room temperature for 5 minutes then centrifuge at 8,000 × *g* for 1 minute.
18. Perform a second elution step using 100 µL Elution Buffer. The user may perform the second elution using the same elution tube or in a different tube.

    Note: The purified DNA is ready to be used in downstream applications or stored at -20°C.

Purify DNA from lignified, polyphenol-rich plant tissues

This protocol describes how to purify DNA from woody, lignified, and/or polyphenol rich samples such as branches, twigs, needles, wax-coated leaves, or wheat flour.

1. In a 1.5 mL microcentrifuge tube, add 350 µL of Lysis Buffer A supplemented with polyvinylpyrrolidone (PVP) at a 2% (w/v) final concentration.
2. Grind up to 100 mg of plant material in liquid nitrogen using a mortar and pestle or grinding mill.
3. Transfer ground plant tissue powder into the tubes with the pre-aliquoted Lysis Buffer A supplemented with PVP.
4. Add 50 µL of Lysis Buffer B and 20 µL RNase A. Mix by vortexing or pipetting.

5. *(Optional)* For tissues resistant to mechanical disruption, add glass sand to the microcentrifuge tube and vortex for 1 minute.
6. Incubate the sample at 65°C for 10 minutes. Vortex occasionally or use a shaking water bath, rocking platform or thermomixer.
7. Add 130 µL of Precipitation Solution then mix by inverting the tube 2–3 times. Incubate on ice for 5 minutes.
8. Centrifuge at ≥20,000 × *g* for 5 minutes. Collect the supernatant (usually 450–550 µL) then transfer to the clean microcentrifuge tube.
9. Add 400 µL of Plant gDNA Binding Solution and 400 µL of 96% ethanol then mix well.
10. Transfer half of the prepared mixture (600–700 µL) to the spin column. Centrifuge at 6,000 × *g* for 1 minute. Discard the flow-through solution then apply the remaining mixture onto the same column.
11. Centrifuge at 6,000 × *g* for 1 minute.

    IMPORTANT! Do not exceed specified relative centrifugal force.

    Note: Close the bag with GeneJET™ Genomic DNA Purification Columns tightly after each use.
12. Add 500 µL of Wash Buffer I to the column. Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through then place the column back into the collection tube.

    Note: Ensure ethanol has been added to Wash Buffer I.
13. Add 500 µL of Wash Buffer II to the column. Centrifuge at ≥20,000 × *g* for 3 mintues.

    Note: Ensure ethanol has been added to Wash Buffer II
14. Empty the collection tube. Place the purification column back into the tube then re-spin the column at ≥20,000 × *g* for 1 minute.
15. Discard the collection tube containing the flow-through solution then transfer the column to a sterile 1.5 mL microcentrifuge tube.
16. To elute genomic DNA, add 100 µL of Elution Buffer to the center of the column membrane. Incubate at room temperature for 5 minutes then centrifuge at 8,000 × *g* for 1 minute.
17. Perform a second elution step using 100 µL Elution Buffer. The user may perform the second elution using the same elution tube or in a different tube.

    Note: The purified DNA is ready to be used in downstream applications or stored at -20°C.

Purify DNA from rapeseeds (Brassica napus)

1. In a 1.5 mL microcentrifuge tube, add 350 µL of Lysis Buffer A supplemented with dithiothreitol (DTT) to a 40 mM final concentration.
2. Grind up to 100 mg of plant material in liquid nitrogen using a mortar and pestle or grinding mill.
3. Transfer the ground plant tissue powder into tubes containing the prealiquoted Lysis Buffer A supplemented with DTT.
4. Add 50 µL of Lysis Buffer B and 20 µL RNase A. Mix by vortexing or pipetting.
5. *(Optional)* For tissues resistant to mechanical disruption, add glass sand to the microcentrifuge tube and vortex for 1 minute.
6. Incubate the sample at 65°C for 10 minutes. Vortex occasionally or use a shaking water bath, rocking platform or thermomixer.
7. Add 130 µL of Precipitation Solution then mix by inverting the tube 2–3 times. Incubate on ice for 5 minutes.
8. Centrifuge at ≥20,000 × *g* for 5 minutes. Collect the supernatant (usually 450–550 µL) then transfer to the clean microcentrifuge tube.
9. Add 400 µL of Plant gDNA Binding Solution and 400 µL of 96% ethanol then mix well.
10. Transfer half of the prepared mixture (600–700 µL) to the spin column. Centrifuge at 6,000 × *g* for 1 minute. Discard the flow-through solution then apply the remaining mixture onto the same column.

11. Centrifuge at 6,000 × *g* for 1 minute.

IMPORTANT! Do not exceed specified relative centrifugal force.

Note: Close the bag with GeneJET™ Genomic DNA Purification Columns tightly after each use.

12. Add 500 µL of Wash Buffer I to the column. Centrifuge at 8,000 × *g* for 1 minute. Discard the flow-through then place the column back into the collection tube.

    Note: Ensure ethanol has been added to Wash Buffer I.

13. Add 500 µL of Wash Buffer II to the column. Centrifuge at ≥20,000 × *g* for 3 mintues.

    Note: Ensure ethanol has been added to Wash Buffer II

14. Empty the collection tube. Place the purification column back into the tube then re-spin the column at ≥20,000 × *g* for 1 minute.

15. Discard the collection tube containing the flow-through solution then transfer the column to a sterile 1.5 mL microcentrifuge tube.

16. To elute genomic DNA, add 100 µL of Elution Buffer to the center of the column membrane. Incubate at room temperature for 5 minutes then centrifuge at 8,000 × *g* for 1 minute.

17. Perform a second elution step using 100 µL Elution Buffer. The user may perform the second elution using the same elution tube or in a different tube.

    Note: The purified DNA is ready to be used in downstream applications or stored at -20°C.

Troubleshooting

Observation	Possible cause	Recommended action
Low yield of purified DNA	Excess sample used during lysate preparation.	Reduce the amount of starting material. Do not use more plant tissue than indicated in lysis protocols.
	Column clogs during procedure.	Ensure the lysate is clear before loading on the spin column. Remove any particulate material by centrifugation.
	Insufficient homogenization of plant material.	To disrupt the cell wall, it is important to homogenize the sample thoroughly until it is ground to a fine powder. Grind up to 100 mg of plant material in liquid nitrogen using a mortar and pestle or grinding mill.
	Ethanol was not added to the lysate.	Ensure ethanol was added to the lysate before applying the sample to the Purification Column.
	Ethanol was not mixed with the lysate.	After the addition of ethanol to the lysate, mix the sample by vortexing or pipetting.
	Ethanol was not added to Wash Buffers.	Ensure ethanol was added to Wash Buffer I and Wash Buffer II before use.
	Incorrect elution conditions.	Following the addition of elution buffer to the column membrane, incubate for 5 minutes prior to centrifugation.
	DNA sample was excessively manipulated.	All pipetting and vortexing steps should be accomplished as gently as possible. Wear gloves to avoid any contamination from DNases.
	Columns stored at room temperature (15–25°C) for longer than 6 months.	Extended room temperature storage (15–25°C) for columns beyond 6 months may lead to membrane drying and reduced DNA yield. For prolonged storage exceeding 6 months, it is recommended to store columns at 2–8°C. See “Contents and storage” on page 1.
Purified DNA is degraded	Sample may be old or degraded.	If possible, use the youngest leaves or tissues. Avoid the leaves and tissues that have been exposed to direct sunlight.
		If samples are stored for future use, flash-freeze in liquid nitrogen and store at -70°C.
RNA contamination	RNase A was not added ot the reaction mixture during lysis.	Add the supplied RNase A to the reaction mixture during lysis. See step 5.
Inhibition of downstream enzymatic reactions	Purified DNA contains residual salt.	Use the correct order for the Wash Buffers. Always wash the purification column with Wash Buffer I first and then proceed to washing with Wash Buffer II.

Documentation and support

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  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

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Revision history: Pub. No. MAN0016131 B00

Revision	Date	Description
B00	17 April 2024	• The document was updated to the current template, with associated updates to the warranty, trademarks, and logos. • The storage conditions for columns and collection tubes were updated.
A.0	12 October 2016	New document for the GeneJET™ Plant Genomic DNA Purification Mini Kit.