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PRODUCT INFORMATION

Thermo Scientific
GeneJET Plant Genomic DNA Purification Mini Kit
#K0791, #K0792

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⚠ Read Storage information (p. 2) before first use!

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For Research Use Only. Not for use in diagnostic procedures.

#__
Lot __
Exp. __

CERTIFICATE OF ANALYSIS

Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit is qualified by isolating genomic DNA from 100 mg of plant tissue following the protocols outlined in the manual. The purified genomic DNA has an $A_{260/280}$ ratio between 1.7 and 1.9. A single band of more than 30 kb is observed after agarose gel electrophoresis and ethidium bromide staining. The functional quality of purified genomic DNA is evaluated by digestion with restriction enzymes.

Quality authorized by: Jurgita Žilinskienė

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COMPONENTS OF THE KIT

GeneJET Plant Genomic DNA Purification Mini Kit	#K0791 50 preps	#K0792 250 preps
RNase A	2 × 0.7 mL	6 × 1.0 mL
Lysis Buffer A	25 mL	120 mL
Lysis Buffer B	3 mL	15 mL
Precipitation Solution	8 mL	40 mL
Plant gDNA Binding Solution	24 mL	120 mL
Wash Buffer I (concentrated)	10 mL	40 mL
Wash Buffer II (concentrated)	10 mL	40 mL
Elution Buffer (10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA)	30 mL	150 mL
GeneJET Genomic DNA Purification Columns pre-assembled with Collection Tubes	50	250
Collection Tubes (2 mL)	50	250

STORAGE

The unopened vial of RNase A solution is stable at room temperature. Once the vial is opened, it should be stored at -20 °C. All other kit components should be stored at room temperature (15-25 °C).

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

DESCRIPTION

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.

PRINCIPLE

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.

IMPORTANT NOTES

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

	#K0791 50 preps		**#K0792** 250 preps	
	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

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

- 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.
- Wear gloves when handling the **Plant gDNA Binding Solution** and **Wash Buffer I** as these reagents contain irritants.
- Typically the purified genomic DNA has an $A_{260/280}$ 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.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and pipette tips
- Vortex
- Ethanol (96-100%)
- 1.5 mL microcentrifuge tubes
- Microcentrifuge
- Thermomixer, shaking water bath or rocking platform capable of heating up to 65 °C
- Disposable gloves
- Liquid nitrogen (with mortar and pestle) or grinding mill
- Polyvinylpyrrolidone (PVP) (with lignified, polyphenol-rich plant tissues)
- Dithiothreitol (DTT) (with rapeseeds)

PROTOCOLS

Protocols for DNA purification from lignified, polyphenol-rich plant tissues and rapeseeds are described on p.6.

A. Plant Genomic DNA Purification Main Protocol

Step	Procedure
1	Pipette 350 µL of **Lysis Buffer A** into 1.5 mL microcentrifuge tube (not provided). Weigh the plant tissue - use up to 100 mg of fresh or frozen tissue; up to 20 mg of lyophilized tissue. Grind the material by one of the following methods: a) Mortar and pestle. Place up to 100 mg of plant tissue into liquid nitrogen and grind thoroughly with a mortar and pestle. b) Grinding mill. 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. Immediately transfer the tissue powder into a 1.5 mL microcentrifuge tube containing 350 µL of Lysis Buffer A. Vortex for 10-20 s 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.
2	Add 50 µL of **Lysis Buffer B** and 20 µL **RNase A**. *Optional: for tissues that are resistant to mechanical disruption, add glass sand to the microcentrifuge tube and vortex for 1 min*.
3	Incubate the sample 10 min at 65 °C vortexing occasionally or use a shaking water bath, rocking platform or thermomixer.
4	Add 130 µL of **Precipitation Solution** and mix by inverting the tube 2-3 times. Incubate 5 min on ice.
5	Centrifuge for 5 min at ≥20,000 × g (≥14,000 rpm).
6	Collect the supernatant (usually 450-550 µL) and transfer to the clean microcentrifuge tube (not provided). Add 400 µL of **Plant gDNA Binding Solution** and 400 µL of 96% ethanol and mix well.

7	Transfer half of the prepared mixture (600-700 µL) to the spin column. Centrifuge for 1 min at 6,000 × g (~8,000 rpm). Discard the flow-through solution and apply the remaining mixture onto the same column. Centrifuge for 1 min at 6,000 × g (~8,000 rpm). ***Important: Do not exceed specified relative centrifugal force.*** **Note.** Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!
8	Add 500 µL of **Wash Buffer I** to the column (ensure ethanol has been added to Wash Buffer I). Centrifuge for 1 min at 8,000 × g (~10,000 rpm). Discard the flow-through and place the column back into the collection tube.
9	Add 500 µL of **Wash Buffer II** to the column (ensure ethanol has been added to Wash Buffer II). Centrifuge for 3 min at maximum speed ≥20,000 × g (≥14,000 rpm). *Recommended: Empty the collection tube. Place the purification column back into the tube and re-spin the column for 1 min. at maximum speed (≥20,000 × g, ≥14,000 rpm).* Discard the collection tube containing the flow-through solution and transfer the column to a sterile 1.5 mL microcentrifuge tube (not provided).
10	To elute genomic DNA, add 100 µL of **Elution Buffer** to the centre of the column membrane, incubate for 5 min at room temperature and centrifuge for 1 min at 8,000 × g (~10,000 rpm).
11	Perform a second elution step using 100 µL **Elution Buffer**. You may perform the second elution using the same elution tube or in a different tube. The purified DNA is ready to be used in downstream applications or stored at -20 °C.

B. DNA Purification from Lignified, Polyphenol-rich Plant Tissues

To purify DNA from woody, lignified and/or polyphenol - rich samples such as branches, twigs, needles, wax-coated leaves (e.g. laurel) and wheat flour, supplement the Lysis Buffer A with polyvinylpyrrolidone (PVP).

Step	Procedure
1	In a 1.5 mL microcentrifuge tube add 350 µL of **Lysis Buffer A** supplemented with PVP at a 2% (w/v) final concentration. Grind up to 100 mg of plant material in liquid nitrogen using a mortar and pestle or grinding mill as described in Step 1 on p.4.
2	Transfer ground plant tissue powder into the tubes with the prealiquoted Lysis Buffer A (with PVP). Add 50 µL of **Lysis Buffer B** and 20 µL **RNase A**. Mix by vortexing or pipetting. *Optional: for tissues resistant to mechanical disruption, add glass sand to the microcentrifuge tube and vortex for 1 min.*
3	Go to step 3 on p.4 of the Plant Genomic DNA Purification Main Protocol.

C. DNA Purification from Rapeseeds (Brassica napus)

Step	Procedure
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. Grind up to 100 mg of plant material in liquid nitrogen using a mortar and pestle or grinding mill as described in Step 1 on p.4.
2	Transfer the ground plant tissue powder into tubes containing the prealiquoted Lysis Buffer A supplemented with DTT. Add 50 µL of **Lysis Buffer B** and 20 µL **RNase A**. Mix by vortexing or pipetting. *Optional: for tissues resistant to mechanical disruption, add glass sand to the microcentrifuge tube and vortex for 1 min.*
3	Proceed to step 3 on p.4 of the Plant Genomic DNA Purification Main Protocol.

TROUBLESHOOTING

Problem	Possible cause and solution
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 as described in Step 1 on p.4. **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. Follow the instructions for Wash Buffer preparation on p.3. **Incorrect elution conditions.** Following the addition of elution buffer to the column membrane, incubate for 5 min 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.
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	Add the supplied RNase A to the reaction mixture during lysis as described in step 2, p.4.
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.

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