

Plant DNA Isolation Kit (Magnetic Bead System) – 50 Preps
Product # 58200

Product Insert

Norgen's Plant DNA Isolation Kit (Magnetic Bead System) provides a rapid method for the isolation and purification of total DNA from a wide range of plant and fungi species. Furthermore, the kit also provides a convenient method for the detection of pathogens which may be infecting a plant, as it allows for the purification of any pathogen DNA along with the purification of the total DNA. Total DNA can be purified from fresh or frozen plant tissues, plant cells or fungi samples using this kit. The DNA is bound to the surface of the magnetic beads under optimized buffer conditions and released using a low salt buffer system. The Plant DNA Isolation Kit (Magnetic Bead System) can be easily adapted to automated magnetic bead separation instruments and work stations. The purified DNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, Southern blotting, SNP analysis and sequencing.

Norgen's Purification Technology

Purification is based on the use of magnetic beads that bind DNA under optimized DNA binding conditions. Plant DNA can either be isolated from fresh or frozen plant samples. The process involves first grinding plant tissue in a mortar with liquid nitrogen (or alternative homogenization equipment). Lysis Buffer L and RNase A are then added, followed by a short incubation at 65°C. Next, Binding Buffer I is added to the lysate followed by another short incubation on ice. The lysate is then spun in order to remove any debris. Ethanol and Magnetic Bead Suspension are then added to the clarified lysate, and the resulting solution is then placed on the magnetic separation rack. Only the DNA will bind to the magnetic beads, while most of the proteins will be removed in the supernatant. The bound DNA is then washed with Wash solution WN and 70% ethanol in order to remove any remaining impurities, and the purified total DNA is eluted with the Elution Buffer B. The purified DNA can be used directly in a number of downstream applications.

Specifications

Kit Specifications	
Number of Preps	50
Maximum Plant Input	50 mg for all types of plant species and tissue
Average Yield from 50 mg of Plant*	3 -10 µg
Average Purity (OD260/280)	1.8 – 2.0
Time to Complete 12 Purifications	50 minutes

* Average DNA yield will vary depending on the sample type

Storage Conditions and Product Stability

Store RNase A at -20°C upon arrival. All other solutions should be kept tightly sealed and stored at room temperature (15 – 25°C). These reagents should remain stable for at least 2 years in their unopened containers.

Advantages

- Fast and easy processing using a magnetic bead system
- Robust lysis system (chemical lysis combined with a mechanical homogenization)
- High yields - Consistent, high yields of inhibitor-free DNA up to 50 kb plus
- Isolate high quality total DNA from a variety of plant species, including any pathogen DNA present

Kit Components

Component	Product # 58200 (50 samples)
Lysis Buffer L	60 mL
RNase A	1 vial
Binding Buffer I	7 mL
Magnetic Bead Suspension	4 x 1.1 mL
Solution WN	18 mL
Elution Buffer B	8 mL
Elution tubes (1.7 mL)	50
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Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at www.norgenbiotek.com.

Solution WN contains guanidinium salts, and should be handled with care. Guanidinium salts forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

Customer-Supplied Reagents and Equipment

- Magnetic bead separation rack
- Micropipettors
- Microcentrifuge tube
- 70% ethanol (prepare fresh)
- 96-100% ethanol
- 65°C Incubator
- Ice
- Liquid nitrogen or any mechanical homogenizer

Procedure

Notes prior to use:

- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- **Always** vortex the **Magnetic Bead Suspension** before use.
- Prepare a working concentration of the **Solution WN** by adding 24 mL of 96 - 100 % ethanol (provided by the user) to the supplied bottle containing the concentrated **Solution WN**. This will give a final volume of 42 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Store the RNase A solution at 4°C for up to 3 months. For longer storage, the RNase A solution should be divided into small aliquots and stored at -20°C.

Flow Chart

Procedure for Purifying Plant DNA using Norgen's Plant DNA Isolation Kit (Magnetic Bead System)

Grind tissue samples using liquid nitrogen.

Transfer ↓



Add Lysis Buffer L and RNase A.
Mix and incubate at 65°C for 10 minutes.
Add Binding Buffer I.
Incubate on ice for 5 minutes.

SPIN ↓



Transfer supernatant. Add Ethanol and
Magnetic Bead Suspension. Mix and
incubate for 5 minutes.



Place tube in magnetic separation rack.
Let stand for 1 minute.

Discard supernatant. Add Solution WN,
mix and incubate for 1 minute.



Place tube in magnetic separation rack.
Let stand for 1 minute.

Discard supernatant. Add 70% ethanol,
mix and incubate for 1 minute.



Repeat ethanol wash step.
Incubate open tube at 65°C for 5 minutes.

Add Elution Buffer B, mix and
Incubate at 65°C for 10 minutes.



Place tube in magnetic separation rack.
Let stand for 1 minute.

Carefully transfer supernatant
to Elution Tube.



Pure Plant DNA

1. Plant Sample Collection and Lysate Preparation

- a. Place ≤ 50 mg of plant tissue into a mortar that contains liquid nitrogen and grind into a powder. Transfer the plant or fungi powder to a DNase-free 1.7 mL microcentrifuge tube (not provided) and add 500 μ L of **Lysis Buffer L** and 1 μ L of **RNase A**.

Alternatively, other homogenization methods can be used with this procedure, including a bead system. If an alternative method is used, add 500 μ L of **Lysis Buffer L** and 1 μ L of **RNase A** to the sample immediately after homogenization and vortex for 20 seconds to mix.
- b. Incubate at 65°C for 10 minutes. Occasionally mix the lysate 2 or 3 times during incubation by inverting the tube.
- c. Add 100 μ L of **Binding Buffer I**, mix by vortexing the tube, and incubate for 5 minutes on ice.
- d. Centrifuge the tube for 2 minutes at 20,000 \times g (~14,000 RPM).
- e. Using a pipette, transfer up to 400 μ L of supernatant (avoid contacting the pellet with the pipette tip) into a 2 mL DNAase-free microcentrifuge tube (not provided).
- f. Add 400 μ L of 96-100% ethanol (provided by the user) and 80 μ L of **Magnetic Bead Suspension** (vortex prior to use) to the lysate collected above. Mix by vortexing.
- g. Incubate at room temperature for 5 minutes. Occasionally invert the tube.
- h. Proceed to Section 2: Plant DNA isolation

2. Plant DNA Isolation

- a. Assemble a magnetic separation rack and place the sample tube in the magnetic rack. Allow to sit for 1 minute.
- b. Aspirate and discard supernatant without touching the magnetic beads.
- c. Remove the sample tube from the magnetic rack and gently add 500 μ L of **Solution WN** (ensure ethanol was added). Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
- d. Place the sample tube on the magnetic rack and allow to sit for 1 minute.
- e. Aspirate and discard supernatant without touching the magnetic beads.
- f. Remove the sample tube from the magnetic rack and gently add 500 μ L of freshly prepared **70% ethanol**. Resuspend by vortexing or pipetting and incubate at room temperature for 1 minute.
- g. Place the sample tube on the magnetic rack and allow to sit for 1 minute.
- h. Aspirate and discard supernatant without touching the magnetic beads.
- i. Repeat **Steps 2f - 2h** for a second wash step.

Note: Remove as much of the 70% ethanol in the sample tube as possible by pipetting.
- j. Incubate the open tube at 65°C for 5 minutes to dry the Magnetic beads.
- k. Remove the sample tube from the magnetic rack and add 75 -100 μ L of **Elution Buffer B**. Mix by vortexing and incubate at 65°C for 10 minute.
- l. Briefly vortex and place sample tube on the magnetic rack and allow to sit for 1 minute.
- m. Carefully transfer the elution to a fresh 1.7 mL elution tube (provided) without touching the magnetic beads. The purified DNA sample may be stored at 4°C for a few days. It is recommended that samples be placed at -20°C for long-term storage

Related Products	Product #
Plant Nucleic Acid Collection and Transport Tubes	45630, 45660
Plant DNA Isolation Kit (50 Prep)	27600
Plant Nucleic Acid Isolation Kit	45600

Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Magnetic beads were accidentally pipetted up with the supernatant.	The pipette tip was placed too close to the magnetic beads while pipetting	Return the magnetic beads and the supernatant back into the sample tube. Mix well, and place the tube back onto the magnetic separation rack for the specified time. Carefully remove the supernatant without touching the magnetic beads.
The yield of genomic DNA is low	Incomplete lysis of cells	Ensure that the homogenization step was done properly. Also longer incubation at 65°C may result in increased yields.
	Amount of magnetic beads added was not sufficient	Prior to use, the magnetic bead suspension has to be well mixed to avoid any inconsistency in DNA isolation.
	Ethanol was not added to the Solution WN	Ensure that 24 mL of 96 - 100% ethanol is added to the supplied Solution WN prior to use.
DNA does not perform well in downstream applications.	Binding Buffer I was not added to the lysate	Ensure that the Binding Buffer I is added to the lysate and that it is incubated on ice for 5 minutes prior to spinning down the lysate.
	DNA was not washed with 70% Ethanol	Traces of salt from the binding step may remain in the sample if the magnetic beads are not washed with 70% Ethanol. Salt may interfere with downstream applications, and thus must be washed from the magnetic beads.
	Ethanol carryover	Ensure that the drying step is performed after the 70% ethanol wash steps in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
RNA is present in eluted DNA.	RNA is coeluted with the DNA.	Carry out a digestion with RNase A on the elution if the RNA present will interfere with downstream applications. Refer to manufacturer's instructions regarding amount of enzyme to use, optimal incubation time and temperature.

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362. Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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