MBead Plant Genomic DNA Kit

Cat No. PDM05-0100
Size: 100 Reactions

Description

This MBead Plant Genomic DNA Kit was designed specifically for genomic DNA isolation from a variety of plant samples. Its unique buffer system will efficiently lyse cells and degrade protein, allowing for DNA to be easily bound by the surface of the magnetic beads. The RNA and other non-specific binding particles are removed with a wash buffer, and the genomic DNA is then released into the Release Buffer. The genomic DNA can be purified manually within 50 minutes (using most magnetic separators) or the kit can be easily adapted to satisfy most automated Nucleic Acid purification systems.

Specifications

- Sample: Up to 50 mg of various plant tissue
- Operation time: Within 50 minutes (manual)
- Applications: Restriction Enzyme Digestion, Southern Blotting, PCR, qPCR and RT-PCR assays
- Storage: Room temperature

Kit Contents

Contents	NA012-0100
Magnetic Bead	2 ml
Lysis Buffer	30 ml
Grind Buffer	40 ml
Wash Buffer	80 ml
Release Buffer	20 ml

Required Materials

- Mortar and pestle
- Liquid nitrogen
- Isopropanol
- Magnetic separator
- 1.5 ml microcentrifuge tubes

MBead Plant Genomic DNA Kit Protocol

Sample Preparation

1. Cut off 100 mg of the fresh plant tissue or 50 mg of the dry plant tissue.
2. Grind the sample in the liquid nitrogen to a fine powder using a mortar and pestle.
3. Add 400 µl of the Grind Buffer to the pestle and mortar and continue to homogenize the sample tissue by grinding.

Step 1 Lysis

1. Transfer the mixture from the Grind Step to a 1.5 ml microcentrifuge tube.
2. Incubate at 70°C for 30 minutes to lyse the sample. During incubation, invert the tube every 5 minutes.
3. Centrifuge for 5 minutes at 5,000 x g.
4. Transfer the supernatant to a new 1.5 ml microcentrifuge tube and add 200 µl of Lysis Buffer. Mix well and incubate at 65°C for 5 minutes. During this time, pre-heat the Release Buffer to 65ºC for Step 4.
5. Add 400 µl of the isopropanol to the lysate and mix well.

Step 2 DNA Binding

1. Add 20 µl of the Magnetic Beads. Mix well by gently shaking for 3 minutes.
2. Place the tube in a magnetic separator for 30 seconds.
3. Remove the solution (If the mixture becomes viscous, increase magnetic bead separation time).

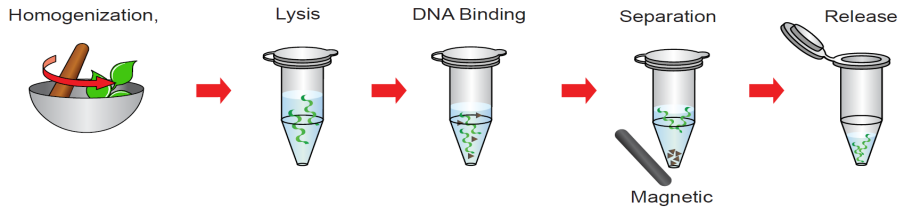
Step 3 Wash

1. Add 800 µl of the Wash Buffer and mix well (Following the wash, the mixture will no longer be viscous).
2. Place the tube in a magnetic separator for 30 seconds. Remove the solution.

Step 4 Release

1. Add 200 µl of the Release Buffer (pre-heated to 65ºC) and mix well.
2. Incubate for 3 minutes at 65ºC (during incubation, shake the tube vigorously every minute).
3. Place the tube in a magnetic separator for 1 minute.
4. Carefully transfer ONLY the clean portion of the solution to a clean tube.

NOTE: Be sure and allow the magnetic beads to disperse completely during the binding, wash and elution



Troubleshooting

Refer to the table below to troubleshooting problems that you may encounter when purifying genomic DNA with the kit.

Problem	Cause	Solution
DNA is sheared or degraded	Lysate mixed too vigorously	Use the appropriate pipette tip set for the required volume, lower it under the reading line of the solution to mix the sample, pipet up and down gently to mix.
	DNase contaminated	Maintain a sterile environment while working (e.g. wear gloves and use DNase-free reagents).
RNA containment	Incomplete removal of the RNase	RNase A treatment
Low yields of gDNA	Incomplete lysis and homogenization	Complete lysis and homogenization. Use the appropriate method for the lysate preparation based on the amount of starting materials. Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in the Lysis Buffer to achieve the optimal lysis.
	Incorrect handling of Magnetic Beads	Vortex the tube containing the Magnetic Beads to fully resuspend the beads before adding them to your sample.
	DNA remains bound to magnetic beads	During the Elution Step, incubate at 65°C for 3 min, and shake the tube vigorously every minute.
	The quality of the starting material may not be optimal.	Use the fresh sample and process immediately after collection, or freeze the sample at -80°C or in the liquid nitrogen.
High background on UV measurement	Residual beads released	Repeat the magnetic separation and transfer eluate to a clean tube.

Related Ordering Information

Cat. No.	Description	Size
MB101-0500	*Taq* DNA polymerase	500 U
MB200-0100	PCR SUPERMIX	100 RXNS
MB201-0100	Hot Start SUPERMIX	100 RXNS
MB25530-0025	Ultrapure Proteinase K	25 mg
AGT001-0500	AGAROSE Tablet, 0.5g	100 tab
LD001-1000	Novel Juice Supplied in 6X Loading Buffer	1 ml
DM003-R500	100 bp DNA Ladder H3 RTU	500 µl
DM010-R500	1 Kb DNA Ladder RTU	500 µl
DM013-R500	XLarge DNA Ladder RTU	500 µl
DN001000	100 mM dNTP Set	4x1 ml
DN001-0250	100 mM dNTP Set	4 x 250 µl
DN025-1000	2.5 mM dNTP Mix	1 ml
DN0010	10 mM dNTP Mix	1 ml

Caution

- Check buffers before use for salt precipitation.
  Re-dissolve any precipitate by warming up to 37°C.
- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.