Genomic DNA Extraction Kit for Plants (HMW, Magnetic Beads)

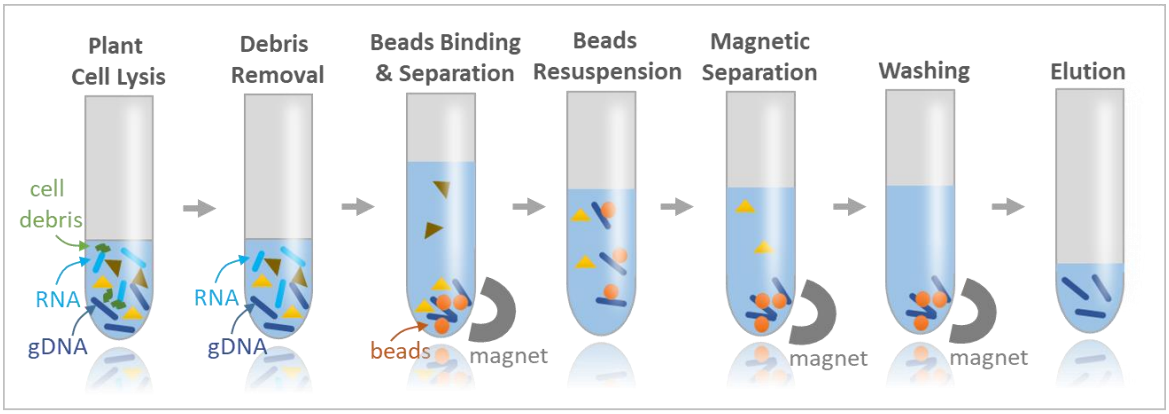
Catalog No.	50021S	50021SP*	50021L	50021LP*
Preps	50 preps	50 preps	200 preps	200 preps

*With Disposable Pestle and Tube Set

Description

The **Genomic DNA Extraction Kit for Plants (HMW, Magnetic Beads)** is a fast and simple reagent for isolating high molecular weight (HMW) genomic DNA from plants using Solid Phase Reversible Immobilization (SPRI) magnetic beads. The magnetic beads-based kit provides a consistent and reliable approach for high-quality plant DNA extraction due to the high-binding capacity and quick magnetic response time.

The extracted plant DNA sizes are above 50 kb and the size ranges are between 50-200 kb. The kit is developed to minimize the common contamination of polysaccharides and polyphenols in plant DNA samples. Purified plant genomic DNA is recovered at high yield and high purity without RNA, polysaccharides, polyphenols, lipids, and salt contaminations. The ratios of A260/A280 are around 1.8, and the ratios of A260/A230 are around 1.5-2.5. Purified HMW plant DNA is suitable for applications such as, long range PCR, restriction enzyme digestion, hybridization assays, long-read sequencing, and other applications.



Plant tissues, such as fresh leaves, seeds, pedals, roots, are homogenized and lysed, then magnetic beads is added to bind plant DNA before the supernatant is removed by magnetic separation. The DNA-binding magnetic beads are washed to remove residual polysaccharides and polyphenols, then resuspend in a buffer followed by magnetic separation to remove supernatant from the DNA-binding beads. The Purified plant DNA is eluted in the Elution Buffer after the washing steps.

Features

- High molecular weight DNA: 50 kb to 200 kb
- High purity
- Simple magnetic beads method
  - No column needed
  - No vacuum needed

	Specifications
Technology	Magnetic beads
Tissue amount	Up to 50 mg
Genomic DNA size	50-200 kb
Genomic DNA yield	Up to 10 µg (dependent on the plant)
Elution volume	100 µl
O.D. 260/280	Around 1.8
O.D. 260/230	Between 1.5-2.5. May have exceptions.

Component

Catalog No.	50021S	50021SP	50021L	50021LP
TE Buffer	0.75 ml	0.75 ml	3 ml	3 ml
Plant Lysis Buffer	18 ml	18 ml	72 ml	72 ml
Proteinase K	150 µl	150 µl	600 µl	600 µl
RNase	150 µl	150 µl	600 µl	600 µl
Plant Beads	7.5 ml	7.5 ml	30 ml	30 ml
GIS Buffer	40 ml	40 ml	160 ml	160 ml
ISP Buffer	15 ml	15 ml	60 ml	60 ml
Elution Buffer	10 ml	10 ml	40 ml	40 ml
Disposable Pestle	-	50	-	200
Disposable Pestle Tube, 1.5 ml	-	50	-	200

Storage Condition

- Store Proteinase K, RNase, Plant Beads, at 4°C, stable up to 12 months.
- Store TE Buffer, Plant Lysis Buffer, GIS Buffer, ISP Buffer, and Elution Buffer at room temperature, stable up to 12 months.

Reagent & Equipment Needed (not provided in this kit)

- Magnetic particle concentrator
- Thermal mixer or heat block
- Vortexer
- 1.5 ml tubes
- Disposable Pestle and Tube Kit (BioDynami Cat. # 50018S and 50018L. Provided for Cat.# 50021SP and 50021LP) or equivalents
- 80% ethanol (prepare before use)
- 100% and 80% isopropanol (prepare before use)

Protocol

Part 1, Homogenization and lysis

1. Prepare a master mix of Plant Lysis Buffer (360 µl per sample), RNase (3 µl per sample), and Proteinase K (3 µl per sample) according to the number of samples.
2. Homogenize 30–50 mg plant sample using a mechanical grinder, or a disposable pestle (provided with Cat.# 50021SP and 50021LP). If working with multiple samples, each sample should be processed quickly.
   - Homogenization with pestles:
     a) Transfer the chopped tissue to a pestle tube, add 15 µl of TE Buffer, and briefly spin down.
     b) Grind the tissue in the tube using the pestle. Leave the pestle in the tube.
     c) Add 360 µl of the above master mix to the tissue, and move the pestle up and down to mix. Make sure no sample materials remain on the pestle and then discard the pestle. If the sample material sticks to the pestle, transfer it into the tube by wiping the pestle tip along the tube rim.
   - Homogenization with a mechanical grinder:
     a) Grind the tissue (following the manufacturer's instructions).
     b) Add 360 µl of the above master mix to the tissue. Vortex to mix thoroughly.
3. Incubate samples in the thermal mixer at 56°C for a minimum of 40 min with agitation. The agitation speed at 1400-2000 rpm is recommended for most applications. Alternatively, incubate samples in a heatblock at 56°C for a minimum of 40 min and tap the tube from time to time to speed up tissue lysis.

Part 2, Genomic DNA extraction

Note: Resuspend Plant beads completely before use.

1. Centrifuge the tubes at 10,000g for 5 min and transfer supernatants to new tubes. DO Not carry over pellets containing cell debris.
2. Add 150 µl of the Plant Beads, mix by inverting the tube 8 times and incubate for 5 min. Put the tube on a magnet rack for 4 min and remove the supernatant using pipette tips. Remove any residue solution using a pipette with fine tips. DO NOT touch the beads.
3. Add 800 µl of GIS Buffer, vortex the tubes briefly to resuspend the beads. Incubate for 2 min.
4. Put the tubes on the magnet rack for 2 min and discard the supernatant. Incubate for 1 min and remove any residue solution using a pipette with fine tips.
5. Add 800 µl of 80% isopropanol, incubate for 2 min (invert the tube several times during incubation). Discard the supernatant. Incubate for 2 min and remove any residue solution using a pipette with fine tips.
6. Add 100 µl of Elution Buffer, remove samples from the magnet, resuspend the beads by tapping the tubes. It may take 2 to 30 min to fully resuspend the beads.
7. Transfer samples to new tubes.
8. Add 300 µl of ISP Buffer to each sample, invert the tube 8 times, and incubate for 3 min.
9. Put the tube on a magnet rack. Incubate for 2 min and carefully remove the supernatant using pipette tips. DO NOT touch the beads.
10. Magnetic beads washing
    1) Add 800 µl of 80% isopropanol, incubate for 5 min (invert the tube several times during incubation). Put samples on the magnet and discard the supernatant.
    2) Add 800 µl of 100% isopropanol, incubate for 5 min at 50°C. Put samples on the magnet and discard the supernatant.
    3) Add 800 µl of 80% ethanol, incubate for 5 min at 50°C. Put samples on the magnet and discard the supernatant. Dry the beads for 1 min and remove any residue solution using a pipette with fine tips.
11. Add 100 µl of Elution Buffer (10 mM Tris, pH 8.0), resuspend the beads by tapping the tubes. It may take 2 to 30 min to fully resuspend the beads. DNA can also be resuspended in TE buffer or Low TE buffer.
12. Put the samples on the magnet and transfer the supernatant (containing DNA) to new tubes.

Troubleshooting

Low DNA concentration

1. Up to 50 mg of tissue can be used. Over 50 mg of tissue will make it difficult to resuspend DNA in solution.
2. For some plant species, 50 mg may cause DNA aggregation and hard to resuspend the magnetic beads. This will result in low yield. Reduce the sample amount if this is the case.
3. Certain types of tissue may have relatively low DNA yield.
4. All tissue should be collected at the bottom of the tube (Part 1, Homogenization and lysis). Make sure no tissue material sticks to the pestle or the wall of the tube when pestle is used.
5. Make sure the tissue is completely homogenized before adding Plant Lysis Buffer. Mix completely after adding the Plant Lysis Buffer.
6. Resuspend Plant Beads completely before use. The Plant Beads is viscous and make sure to add an accurate volume of Plant Beads.
7. Make sure the beads are fully resuspended in the buffer (step 6 and 11). Extend the time if needed.

Low O.D. 260/230

1. Discard supernatant completely when washing (step 4, 5, and 10).
2. Remove any residue solution using a pipette with fine tips at step 4, 5, and 10.

Quality Control

Kit components passed stringent functional quality test.

Product Use Limitation

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