

【Product Name】 MagPure Seed DNA Precast Kit (Auto Pure 96)

【Product Specification】 96 Preps/Kit

【Intended Use】

MagPure Seed DNA Kit supplies a simple and rapid extraction of genomic DNA from different plant pieces and seed. The kit is based on superparamagnetic particles purification technology, no phenol-chloroform extraction or alcohol precipitation. The whole extraction process is only 60 minutes. This kit can use on different automatic extraction machines like KingFisher ML, KingFisher Flex and KingFisher Duo. Purified DNA can be used directly for PCR, quantitative PCR, Southern Blot, hybridization, and transgenosis detection

【Principle】

This product is based on the purification method of high binding magnetic particles. Samples are first mechanically disrupted and then chemically lysed. After adding magnetic particles and binding solution, DNA will be adsorbed on the surface of magnetic particles, and impurities such as proteins will be removed without adsorption. The adsorbed particles were washed with washing buffer to remove proteins and impurities, washed with ethanol to remove salts, and finally DNA was eluted by Elution Buffer.

【Main Composition】

| Product | Contents and volume | D6352-F-96 |
|-------------------------|--|------------|
| Purification times | | 90 Preps |
| Buffer SOL | | 90 ml |
| Buffer SDS | | 9 ml |
| 96-Tip (AS) | | 1 |
| Sample Plate (DW Plate) | 600µl Buffer MPB | 1 |
| Wash Plate 1 (DW Plate) | 600µl Buffer GW1 | 1 |
| Wash Plate 2 (DW Plate) | 600µl Buffer GW1 | 1 |
| Wash Plate 3 (DW Plate) | 30µl MagPure Particles , 600µl Buffer GW2 | 1 |
| Wash Plate 4 (DW Plate) | 600µl Buffer GW2 | 1 |
| Elute Plate | 100µl Elution Buffer | 1 |

【Storage conditions and validity】

This kit is shipped and stored at room temperature and is valid for 18 months.

【Applicable Instrument】

Nucleic Acid Extraction Machine such as Auto Pure 96 (Allsheng), Magmix 96 and other similar.

【Part 1: Sample Preparation】

A: Plant Seed (high yield):

1. The Plant seeds are ground into fine powder by high speed grinder and transfer 30-100 mg of the powders to 2ml microcentrifuge tube.
2. **Add one 3 mm tungsten carbide bead to the tube and add 0.8ml Buffer SOL to the sample.** Vortex at high speed for 10 min or Place the tubes into tissuelyser or GeneGrinder. Homogenize the samples for 3~5 min at 30~50 Hz. Proceed step 3.
3. Incubate the mixture for 10 min at 65°C. Centrifuge the lysate for 5 min at >10,000 x g.

B: Fast Protocol (young leaf/fruit):

1. Place a tender sample (young leaf or fruit) into a 2ml safe-lock microcentrifuge tube containing beads (one 3mm tungsten). If processing fresh or frozen plant tissue, 50 mg of starting material is sufficient.
2. Add 0.7ml Buffer SOL to the sample and place the tubes into tissuelyser or GeneGrinder. Homogenize the samples for 3-10min at 30~50 Hz.
3. Incubate the mixture for 10 min at 65°C. Centrifuge the lysate for 5 min at >10,000 x g.

C: Difficult to grind sample Protocol:

1. Disrupt 50 mg plant or fungal tissue by bead-beat methods.
2. Add 0.6ml Buffer SOL to the sample and homogenize the samples for ~2 min at 30-50HZ.
3. Incubate the mixture for 10 min at 65°C. Centrifuge the lysate for 5 min at >10,000 x g.

D: SDS Lysis Protocol(High weight genomic DNA):

1. Disrupt plant or fungal tissue by Liquid nitrogen ground or other bead-beat methods.

2. Add 500µl Buffer SOL and 50µl Buffer SDS to a maximum of 50 mg (wet weight) or 15 mg (dried) disrupted plant or fungal tissue and vortex vigorously.
3. Incubate the mixture for 10 min at 65°C. Centrifuge the lysate for 5 min at >10,000 x g.

【Part 2: Auto Pure 96 nucleic acid extractor operation】

1. Take out the required components of the kit.
2. Inverting the Plate several times to re-suspend the magnetic beads. Pat the plate to make reagents fall back to the bottom of plate.
3. Stay the plate at table for 1 minute, remove the sealing pack and sealing film
4. Add 400µl samples (Step 3 from Part 1) into the Sample Plate.
5. Insert the 96- tip (AS-Tip) into Wash Plate 3. Place the plates on the machine. Turn on the machine and start the D6352-F-96 protocol.
6. After the extraction complete ~ 30 minutes, remove the 96 well plate and 96 tip.
7. Transfer the purified DNA into a new 1.5ml centrifuge tube and store at -20~-8 °C.

【Auto Pure 96 program recommendation】

| Name | Plate Name | Plate | Mix time (sec) | Mix 1-100% | Wait | Volume (ul) | Mix Speed (1-10) | Magnet (0-5) | Repeat (1-10) | Magnet Speed (1-10) | Stay (min) | Hover (min) | 1 st Step Magnet time | 2 nd step Magnet time | 3 rd step Magnet time |
|---------------|---------------|-------|----------------|------------|-------|-------------|-------------------|--------------|---------------|---------------------|------------|-------------|----------------------------------|----------------------------------|----------------------------------|
| 96-Tip | 96-Tip/Wash 3 | 4 | 0 | 0 | 0 | 0 | | | | | | | | | |
| Collect Beads | Wash Plate 3 | 4 | 30 | 70% | 0 | 600 | 8 | 3 | 1 | 5 | 0 | 0 | 3 | 3 | 3 |
| Bind DNA | Sample Plate | 1 | 300 | 70% | 0 | 1000 | 8 | 3 | 1 | 5 | 0.5 | 0 | 5 | 5 | 5 |
| Wash 1 | Wash Plate 1 | 2 | 90 | 70% | 0 | 600 | 8 | 3 | 1 | 2 | 0 | 0 | 3 | 3 | 3 |
| Wash 2 | Wash Plate 2 | 3 | 90 | 70% | 0 | 600 | 8 | 3 | 1 | 2 | 0 | 0 | 5 | 5 | 5 |
| Wash 3 | Wash Plate 3 | 4 | 60 | 70% | 0 | 600 | 8 | 3 | 1 | 1 | 0 | 0 | 3 | 3 | 3 |
| Wash 4 | Wash Plate 4 | 5 | 60 | 70% | 0 | 600 | 8 | 3 | 1 | 1 | 0 | 0 | 3 | 3 | 3 |
| Dry | Elute Plate | 6 | 0 | 0 | 5 min | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Elute | Elute Plate | 6 | 400 | 70% | 0 | 100 | 9 | 3 | 1 | 5 | 0 | 0 | 3 | 3 | 3 |
| Drop | Wash Plate 3 | 4 | 30 | 70% | 1 min | 600 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |