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Magbead Plant Nucleic Acid(DNA/RNA) Extraction Kit

Product Number: DNK81

Shipping and Storage

The reagent kit is stored at 4~30℃ and has an expiration date of 18 months.

Components

For use with 96-throughput Automated Extractors

Component	DNK81 96Preps
Lysis-Binding Buffer Plate	96 Preps
Washing Buffer1 Plate	96 Preps*2
Washing Buffer2 Plate	96 Preps
Magnetic Beads-Washing Plate	96 Preps
Elution Buffer Plate	96 Preps
LPM Buffer	75 mL
Elution Buffer	1.2 mL
96-Magnetic sleeve	96 Preps
RNase A	1.2 mL

For use with 32-throughput Automated Extractors

Component	DNK81 96Preps	DNK81 32Preps
Pre-packed Plate	16 Preps*6	16 Preps*2
8-Magnetic sleeve	2 Sleeves*6	2 Sleeves*2
LPM Buffer	75 mL	25 mL
Elution Buffer	1.2 mL	1.2 mL
RNase A	1.2 mL	0.4 mL

For use with Magnetic Racks

Component	DNK81 100Preps
Lysis-Binding Buffer	66 mL
Washing Buffer1	110 mL
Washing Buffer2	110 mL
Elution Buffer	16 mL
Magnetic Beads	2.4 mL
LPM Buffer	80 mL
RNase A	1.2 mL

Note:1) You can choose 8preps or 16preps of pre packaged Plate

2)The components of different models of test kits cannot be interchanged, and the components of test kits with different batch numbers cannot be interchanged. In order to minimize the absorbance effect of blank solution, when measuring nucleic acid concentration (A260, A280, A230) using UV absorbance method, Buffer EB is used as the blank control.

Description

This reagent kit uses magnetic beads with unique separation properties and a unique buffer system to isolate and purify high-quality DNA from plant seed samples. The reagent kit undergoes steps such as magnetic bead and nucleic acid binding, cleaning,

and elution to remove other impurities in proteins. The resulting DNA product has high purity and can be directly applied in various downstream molecular biology experiments such as qPCR, library construction, and NGS.

This product is perfectly compatible with an automatic nucleic acid extractor, which uses magnetic rods to adsorb, transfer, and release magnetic beads, thereby achieving the transfer of magnetic beads and nucleic acids, improving the degree of automation. The entire experimental process is safe and convenient, with high purity of extracted DNA and no contamination of proteins and other impurities.

Application

1. Used for the extraction, enrichment, and purification steps of nucleic acids, this product is only for scientific research use.
2. This product is suitable for rapid extraction of DNA from plant seed samples, and the processed products can be used for scientific research.

Sample requirements

Applicable specimen types: plant seed samples

Note

1. Please read this manual carefully before the experiment.
2. To avoid any potential biological hazards in the sample, the test sample should be considered infectious and avoid contact with skin and mucous membranes; The handling of samples is recommended to be carried out in a biosafety cabinet that can prevent the outflow of mist. The test tubes and suction heads used in the sample preparation area must be placed in containers containing disinfectants and sterilized together with the waste before being discarded; Sample operation and processing must comply with relevant regulatory requirements: the General Guidelines for Biological Safety in Microbial Biomedical Laboratories and the Regulations on Medical Waste Management issued by the Ministry of Health.
3. The components in the reagent kit must be used within the validity period. Failure to use the components provided in this reagent kit for experiments may result in incorrect results.
4. Laboratory management should strictly follow the management standards of PCR gene amplification laboratories. Experimental personnel must receive professional training, and the experimental process should be strictly divided into zones (reagent preparation zone, sample preparation zone, amplification and product analysis zone). Consumables used should be sterilized and used in one go. Specialized instruments and equipment should be used in each stage of the experimental operation, and supplies in each zone and stage should not be used interchangeably.
5. Use disposable centrifuge tubes and pipettes sterilized under high pressure or purchase centrifuge tubes and pipettes without DNA/RNA enzymes.
6. After completing the nucleic acid extraction of the sample, it is recommended to proceed to the next step of the experiment immediately. Otherwise, please store it at -20 ℃ for use (within 24 hours).
7. After the experiment is completed, treat the workbench and pipette with 5% hypochlorous acid or 75% alcohol, and then irradiate with a UV lamp for 20-30 minutes.

Protocol(Automated operation steps-)

Manual extraction operation

1. Sample lysis
   1.1. **Liquid nitrogen grinding method:** Grind plant seed samples into powder using liquid nitrogen, transfer 30-50 mg of powder into a 2 mL centrifuge tube, add 700uL LPM Buffer, vortex and mix well to fully disperse the sample, and incubate at 65 ℃ for 20 minutes.
   1.2. **Ball mill lysis method:** Grind the sample into fine powder using liquid nitrogen (wet sample) or a powder mixer (dry sample), transfer 30-50 mg of powder into a 2 mL grinding tube, add 3-5 grinding beads and 700 uL LPM buffer, transfer to a grinder for grinding and homogenization, and incubate at 65 ℃ for 20 minutes.

1.3. **Wet grinding method:** Transfer 50-100 mg of plant seed samples into a 2 mL grinding tube, add 3-5 grinding beads and 700 uL LPM Buffer, transfer and use a grinder to grind the homogenate, and incubate at 65 ℃ for 20 minutes.

Note: When using grinding beads, the size and quantity of the beads, as well as the grinding time and power, can affect the yield and fragmentation of DNA. Therefore, appropriate adjustments should be made based on the brand and experimental results of the grinder.

2. After incubation, add 10ul of RNase A, vortex mix well, and leave at room temperature for 10 minutes.
3. Centrifuge at 12000x g for 4 minutes, transfer 500ul of supernatant to a new 2 mL centrifuge tube.
4. Add 20ul Magnetic Beads and 600ul Lysis Binding Buffer to the centrifuge tube, vortex at 800rpm and room temperature for 5 minutes.
5. Place the centrifuge tube on a magnetic frame for 30 seconds, so that the magnetic beads inside the tube are completely adsorbed onto the wall of the centrifuge tube. Use a pipette to remove the liquid inside the tube and remove the centrifuge tube.
6. Add 500ul of Washing Buffer 1, vortex and mix for 1 minute to suspend the magnetic beads. Use a magnetic rack to adsorb the magnetic beads. After 30 seconds, remove the liquid from the tube and remove the centrifuge tube.
7. Add 500ul of Washing Buffer 1, vortex and mix for 1 minute to resuspend the magnetic beads evenly. Use a magnetic rack to adsorb the magnetic beads, and after 30 seconds, remove the liquid from the tube and remove the centrifuge tube.
8. Add 500ul of Washing Buffer 2, vortex and mix for 1 minute to resuspend the magnetic beads evenly. Use a magnetic rack to adsorb the magnetic beads, and after 30 seconds, remove the liquid from the tube and remove the centrifuge tube.
9. Add 500ul of Washing Buffer 2, vortex and mix for 1 minute to resuspend the magnetic beads evenly. Use a magnetic rack to adsorb the magnetic beads, and after 30 seconds, remove the liquid from the tube and remove the centrifuge tube.
10. Collect droplets on the wall of the tube through brief centrifugation, use a magnetic frame to adsorb magnetic beads, and after 30 seconds, remove all liquids from the tube.
11. Open the lid and let it dry in a 40 ℃ metal bath for about 5 minutes (until the surface of the magnetic beads is dull).

Note: Residual ethanol can inhibit subsequent enzyme reactions. When drying, ensure that ethanol evaporates completely. Prolonged drying can lead to difficulty in eluting nucleic acids.

12. Add 50-80uL Elution Buffer, vortex to resuspend the magnetic beads, and incubate at 800rpm and 55 ℃ for 7 minutes.
13. Collect droplets on the wall of the centrifuge tube through brief centrifugation. Place the centrifuge tube on a magnetic rack for 30 seconds to allow the magnetic beads to be adsorbed. Transfer the liquid to a clean 1.5 mL centrifuge tube for later use. The purified nucleic acid can be used for downstream analysis. If not in a hurry to use, the nucleic acid solution can be stored in a -20 ℃ refrigerator for later use.

Automatic nucleic acid extractor operation

Strip pre packaged reagent extraction plan (note: only the first column of the 8-reaction pre packaged reagent board is Lysis Binding Buffer Plate)

1. Sample pre-treatment: The pre-treatment steps for plant seed samples are the same as steps 1-3 of the manual method. After the pre-treatment steps are completed, they are used as backup samples for testing.
2. Invert the pre packaged plate placed at room temperature three times and centrifuge briefly (or shake by hand) in a 96 well plate centrifuge to avoid liquid hanging. Tear open the sealing aluminum foil film on the Pre packaged Plate and confirm the direction of the extraction plate (magnetic bead liquid in columns 4/10).
3. Transfer 500uL of the test sample to the Lysis Binding Buffer Plate (columns 1/7).
4. Set extraction program: (Suitable for the heated bar nucleic acid extractor in column 6)

Step	Hole	Name	Waiting Time(s)	Mixing Time(s)	Magnetization Time(s)	Mixing Speed 1-8	Volume μL	Temperature state	Temperature ℃
1	4	Magnetic shift	0	20	20	8	500	Off	0
2	1	Combination	0	300	20	5	1000	Off	0
3	2	Wash 1	0	90	20	8	500	Off	0
4	3	Wash 1	0	90	20	8	500	Off	0
5	4	Wash 2	0	60	20	8	500	Off	0

6	5	Wash 2	0	60	20	8	500	Off	0
7	6	Elution	300	400	20	5	80	6th hole	55
8	4	Demagnetization	0	10	0	8	500	Off	0

5. Put the pre packaged Plate and 8-Magnetic sleeve that have been added to the sample into the automatic nucleic acid extractor and start the program.
6. After extraction, the purified nucleic acid is in column 6/12. Carefully transfer the nucleic acid to a clean 1.5 mL centrifuge tube using a pipette for later use. The purified nucleic acid can be used for downstream analysis. If not in a hurry to use, the nucleic acid solution can be stored in a -20 ℃ refrigerator for later use.

Note: If the magnetic absorption parameter of the extractor is the number of magnetic absorption times, the time is 20 seconds per time.

Plate pre packaged reagent extraction scheme

1. Sample pre-treatment: The pre-treatment steps for plant seed samples are the same as steps 1-3 of the manual method. After the pre-treatment steps are completed, they are used as backup samples for testing.
2. Invert the pre installed plate placed at room temperature three times and centrifuge briefly (or shake by hand) in a 96 well plate centrifuge to avoid liquid hanging.
3. Tear open the sealing aluminum foil film of the Lysis Binding Buffer Plate, transfer 500 μ L of the sample to be tested into the Lysis Binding Buffer Plate, and place the Lysis Binding Buffer Plate with the added sample in position 1 of the extractor.
4. Tear open the sealing aluminum foil film of the Magnetic Beads Washing Plate and place it in position 4 of the extractor.

(Note: In order to prevent the magnetic bead liquid from hanging on the film and sticking to the hole wall, please make sure to invert and mix the aluminum foil film three times before tearing it off. Then, vigorously shake the bottom of the plate downwards twice, so that all the liquid in each hole slides down to the bottom of the hole before tearing off the aluminum foil film.)

5. Tear open the sealing aluminum foil film of the Washington Buffer1 Plate and place two Washington Buffer1 Plates in positions 2 and 3 of the extractor.
6. Tear open the sealing aluminum foil film of the Washington Buffer2 Plate and place it in position 5 of the extractor.
7. Tear open the sealing aluminum foil film of the Elution Buffer Plate and place it in position 6 of the extractor.

(Note: The liquid volume of the Solution Buffer Plate is small. In order to prevent the liquid from hanging on the sealed aluminum foil film, please make sure that the bottom of the plate is facing downwards before tearing off the aluminum foil film. Shake twice with force to make all the liquid in each hole slide to the bottom of the hole before tearing off the aluminum foil film.)

8. Set extraction program: (Suitable for plate nucleic acid extractor heated on the 6th plate)

Step	Hole	Name	Waiting Time(s)	Mixing Time(s)	Magnetization Time(s)	Mixing Speed 1-8	Volume μL	Temperature state	Temperature ℃
1	4	Magnetic shift	0	20	20	8	500	Off	0
2	1	Combination	0	300	20	5	1000	Off	0
3	2	Wash 1	0	90	20	8	500	Off	0
4	3	Wash 1	0	90	20	8	500	Off	0
5	4	Wash 2	0	60	20	8	500	Off	0
6	5	Wash 2	0	60	20	8	500	Off	0
7	6	Elution	300	400	20	5	80	6th hole	55
8	4	Demagnetization	0	10	0	8	500	Off	0

9. Insert the 96 well magnetic rod sleeve into the automatic nucleic acid extractor and start the program.
10. After extraction, the purified nucleic acid is placed on plate 6 and carefully transferred to a clean 1.5 mL centrifuge tube using a pipette for later use. The purified nucleic acid can be used for downstream analysis. If not in a hurry to use, the nucleic acid solution can be stored in a -20 ℃ refrigerator for later use.

Note: If the magnetic absorption parameter of the extractor is the number of magnetic absorption times, time=20 seconds/time

Limitations of the product

The efficiency of sample extraction is related to whether the operator strictly follows the instructions.

Product performance indicators

1. Appearance inspection: The appearance of each component is clean, without leakage or damage; The label should be complete and undamaged, the identification should be clear and complete, and there should be no omission of information; Visually, RNase A is a colorless and transparent liquid, clear and free of impurities; Visually, when the magnetic bead solution and magnetic bead washing solution are mixed, they are uniformly brown black and have no precipitate. After standing, the magnetic beads precipitate into brown black, and the supernatant is clear and transparent.
2. The ratio of A260/A280 extracted from this reagent kit is between 1.7 and 2.1.