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Magbead Fungal DNA mini extraction kit

Product Number: DNK77MB-96

Description

This kit uses magnetic beads with unique separation capabilities and a unique buffer system to isolate and purify high-quality DNA from various cultured and parasitic fungal samples. The reagent kit undergoes steps such as magnetic bead binding with nucleic acid, cleaning, and elution to remove protein and other impurities. The resulting DNA product has high purity and can be directly applied to various downstream molecular biology experiments such as qPCR, library construction, NGS, etc. This product can be perfectly matched with an automatic nucleic acid extractor. By adsorbing, transferring, and releasing magnetic beads with a magnetic rod, the transfer of magnetic beads and nucleic acids can be achieved, improving the degree of automation. The entire experimental process is safe and convenient, and the extracted DNA has high purity without contamination from proteins and other impurities.

Application

This product is suitable for rapid extraction of DNA from various cultured fungal and parasitic fungal samples, and the processed products can be used for scientific research. This product is only for scientific research purposes and is used for the extraction, enrichment, and purification steps of nucleic acids.

Components

Pre packaged: 32 reaction/box, 48 reaction/box, 64 reaction/box (note: 8-reaction or 16 reaction pre packaged reagent plates can be selected)

Component	Specifications		
	32 Reaction/Box	48 Reaction/Box	64 Reaction/Box
8 Reaction pre packaged reagent board	4 pieces	6 pieces	8 pieces
16 Reaction pre packaged reagent board	2 pieces	3 pieces	4 pieces
8 Joint magnetic rod cover	4 pieces	6 pieces	8 pieces
SACP liquid	22mL/Bottle	32mLBottle	42mL/Bottle
NSH liquid	7mL/Bottle	10.5mL/Bottle	13.5mL/Bottle
Eluent	2mL/Tube	2mL/Tube	2mL/Tube
SURBEAD	1 Reaction/Tube*32	1Reaction/Tub*48	1Reaction/Tub*64
Settling agent	1.1mL/Tube	1.8mL/Tube	1.1mL/Tube*2
Protease K solution	0.7mL/Tube	1.2mLTube	1.4mL/Tube
RNase A	0.4mL/Tube	0.6mL/Tube	0.7mL/Tube

Pre packaged: 96 reactions per box

Component	Specifications	Quantity
Combination liquid pre installation plate	96 Reaction/Plate	1piece
Magnetic bead washing solution pre installed plate	96 Reaction/Plate	1piece
Washing solution 1 pre installed plate	96 Reaction/Plate	2pieces
Washing solution 2 pre installed plate	96 Reaction/Plate	1piece
Pre loaded eluent plate	96 Reaction/Plate	1piece
SACP liquid	63mL/Bottle	1Bottle
NSH liquid	21mL/Bottle	1Bottle
SURBEAD	1 Reaction/Tube	96Tubes
Eluent	2mL/Tube	1Tube
96 hole magnetic rod sleeve	96 Reaction/Plate	1piece

Settling agent	3.1mL/Bottle	1Bottle
Protease K solution	1.2mL/Tube	2Tubes
RNase A	1.1mL/Tube	1Tube

Large packaging: 50 reactions per box

Component	Specifications	Quantity
Binding Buffer	33mL/Bottle	1Bottle
Magnetic bead solution	1.2mL/Tube	1Tube
Washing solution 1	75mL/Bottle	1Bottle
Washing solution 2	75mL/Bottle	1Bottle
Eluent	12mL/Bottle	1Bottle
SACP liquid	33mL/Bottle	1Bottle
NSH 液	12mL/Bottle	1Bottle
NSH liquid	1.8mL/Tube	1Tube
SURBEAD	1Reaction/Tube	50Tubes
Protease K solution	1.2mL/Tube	1Tube
RNase A	0.6mL/Tube	1Tube

Note: The components of different types of reagent kits cannot be interchanged, and the components of different batch numbers of reagent kits cannot be interchanged. In order to minimize the impact of the absorbance of the blank solution, the UV absorbance method is used to determine the nucleic acid concentration (A260, A280, A230) using eluent as the blank control.

Storage conditions and expiration date

1. The reagent kit is stored at 4~30℃ and has an expiration date of 18 months.
2. If there is precipitation in the SACP solution, it can be preheated in a 37℃ water bath for 10 minutes before use to dissolve the precipitate without affecting the effect.
3. After opening, store NSH, precipitant, and proteinase K solution at 4℃.

Sample requirements

Applicable specimen types: various cultured fungal and parasitic fungal samples.

Protocol

1. Preparation before the experiment

Prepare impurity removal solution (currently prepared and used), and prepare it for individual samples using impurity removal solution (the impurity removal solution should be stored at 2-8℃).

The preparation method is: 200μL NSH solution+30μL precipitant (invert and shake well before use). (Calculate and prepare the total volume of impurity removal solution required for this experiment based on the amount of impurity removal solution and the number of samples for each individual sample mentioned above.)

2. Manual extraction operation

2.1. Sample Collection

2.1.1. Solid culture fungi: Take fungal samples from the culture medium as ground samples.

2.1.2. Fungi cultured in liquid form: Collect fungi by centrifugation or filtration, and try to remove the culture medium as much as possible. Use the collected mycelium as a ground sample.

2.1.3. Fungal parasitic fungi: Directly use the sample as a ground sample.

2.2. Sample Grinding (Note: It is recommended to use the recommended amount of sample first, as excessive sample can cause column blockage, thereby affecting yield and purity. For the initial experiment, it is recommended to use 20mg of sample, and adjust the sample dosage according to the experimental process and results)

2.2.1. Liquid Nitrogen Grinding: Transfer the ground sample to a mortar, add liquid nitrogen to grind it into fine powder, weigh ≤ 50 mg of powdered fungi into a new centrifuge tube, and add 600μL SACP solution and 20μL proteinase K solution.

2.2.2. Bead Grinding: Weigh ≤ 50 mg of ground sample into SURBEAD, add 600μL of LSACP solution and 20μL of proteinase K solution, and grind using a grinder (grind at a frequency of 60 Hz or 6M/S for 30 seconds, with a 30 second interval, for a total of 2 cycles).

2.3. Place the mixed solution sample at 800rpm and shake at 56℃ for 30 minutes. Centrifuge at 15000kg for 1 minute, transfer all supernatant to a new 2mL centrifuge tube.

2.4. Add 10μL RNase A, vortex and mix well, and let it stand at room temperature for 10 minutes.

2.5. Add 230μL of impurity removal solution (shake and absorb before use), mix well, vortex for 5 seconds, stand at 4℃ for 10 minutes, centrifuge at 15000xg for 5 minutes, discard the precipitate, and transfer all supernatant to a new 2mL centrifuge tube.

2.6. Add an equal volume of the supernatant obtained above to the centrifuge tube, vortex and mix well.

2.7. Transfer the mixture from step 6 to a new 2mL centrifuge tube for later use (the total volume of the mixture should not exceed 1.2mL).

2.8. Add 20μL of magnetic bead solution to the centrifuge tube, invert and mix well for 5 minutes.

2.9. Place the centrifuge tube on the magnetic rack for 2 minutes to allow the magnetic beads inside the tube to be completely adsorbed onto the centrifuge tube wall. Use a pipette to remove the liquid inside the tube and remove the centrifuge tube.

2.10. Add 700μL of washing solution 1, vortex and mix for 3 minutes 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.

2.11. Add 700μL of washing solution 1, vortex and mix for 3 minutes to resuspend the magnetic beads evenly. Use a magnetic rack to adsorb the magnetic beads. After 30 seconds, remove the liquid from the tube and remove the centrifuge tube.

2.12. Add 700μL of washing solution 2, vortex and mix for 3 minutes to resuspend the magnetic beads evenly. Use a magnetic rack to adsorb the magnetic beads. After 30 seconds, remove the liquid from the tube and remove the centrifuge tube.

2.13. Add 700μL of washing solution 2, vortex and mix for 3 minutes to resuspend the magnetic beads evenly. Use a magnetic rack to adsorb the magnetic beads. After 30 seconds, remove the liquid from the tube and remove the centrifuge tube.

2.14. Collect liquid droplets on the wall of the tube by brief centrifugation, use a magnetic frame to adsorb magnetic beads, and remove all liquid inside the tube after 30 seconds.

2.15. Open the lid and dry in a 40℃ metal bath for about 5 minutes (until the surface of the magnetic beads becomes dull).
Attention: Ethanol residue can inhibit subsequent enzyme reactions. When drying, make sure that the ethanol evaporates completely. Prolonged drying can make it difficult for nucleic acids to be eluted.

2.16. Add 50-70μL of eluent, vortex to resuspend the magnetic beads, and incubate at 56℃ for 5 minutes.

2.17. Collect liquid droplets on the wall of the centrifuge tube by 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 1.5mL 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.

3. Operation of automatic nucleic acid extractor

3.1. Bar style pre packaged reagent extraction scheme (Note: Only column 1 of the 8-reaction pre packaged reagent plate is the binding solution well)

3.1.1. Sample pretreatment: The pretreatment steps for fungal samples are the same as the manual method steps 1-5. After the pretreatment steps are completed, they are used as backup samples for testing.

3.1.2. Invert the pre packaged reagent plate placed at room temperature three times and centrifuge briefly (or shake by hand) in a 96 well centrifuge to avoid liquid accumulation. Tear off the sealing aluminum foil film on the pre packaged reagent board and confirm the direction of the extraction board (magnetic bead solution in column 4/10).

3.1.3. Transfer the test sample (transfer sample size not exceeding 500μL) to the binding solution well (column 1/7).

3.1.4. Set extraction program: (Suitable for the 6th column heated strip nucleic acid extractor)

Step	Hole position	Name	Waiting time (seconds)	Mixing time (seconds)	Magnetic attraction time (seconds)	Mixing speed 1~8	Volume μL	Temperature status	Temperature ℃
1	4	Magnetic transfer	0	20	25	8	700	Close	0
2	1	Combine	0	300	60	7	500	Close	0
3	2	Wash	0	0	0	8	700	Close	0
4	1	Combine	0	10	60	8	500	Close	0
5	2	Wash	0	0	0	8	700	Close	0
6	1	Combine	0	10	60	8	500	Close	0
7	2	Wash	0	0	25	8	700	Close	0
8	3	Wash	0	0	25	8	500	Close	0
9	4	Wash	0	0	25	8	700	Close	0
10	5	Wash	0	0	25	8	500	Close	0
11	6	Elution	300	300	25	5	70	The 6th hole	56
12	4	Abandoning magnetism	0	10	0	8	700	Close	0

3.1.5. Place the pre packaged reagent plate and 8-link magnetic rod sleeve that have been added to the sample into the automatic nucleic acid extractor and start the program.

3.1.6. After extraction, the purified nucleic acid is in column 6/12. Carefully transfer the nucleic acid to a clean 1.5mL 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 attraction parameter of the extractor is the number of magnetic attraction times, the time is 20 seconds per time.

3.2. Plate pre packaged reagent extraction plan

3.2.1. Sample pretreatment: The pretreatment steps for fungal samples are the same as the manual method steps 1-5. After the pretreatment steps are completed, they are used as backup samples for testing.

3.2.2. Invert the pre installed plate placed at room temperature three times and centrifuge briefly (or shake by hand) in a 96 well centrifuge to avoid liquid accumulation.

3.2.3. Tear off the sealing aluminum foil film of the binding solution pre installation plate, transfer the test sample (with a transfer sample volume not exceeding 500μL) to the binding solution pre installation plate, and place the pre installation plate with added samples in the No.1 position of the extractor.

3.2.4. Tear off the sealing aluminum foil film of the magnetic bead washing solution pre installed plate, and place the magnetic bead washing solution pre installed plate 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, before tearing off the aluminum foil film, be sure to mix the board upside down three times, and then shake the board bottom downwards twice with force, so that all the liquid in each hole slides down to the bottom of the hole before tearing off the aluminum foil film.)

3.2.5. Tear off the sealing aluminum foil film of the pre installed detergent 1 plate, and place the two pre installed detergent 1 plates in positions 2 and 3 of the extractor.

3.2.6. Tear off the sealing aluminum foil film of the pre installed plate of detergent 2, and place the pre installed plate of detergent 2 in position 5 of the extractor.

3.2.7. Tear off the sealing aluminum foil film of the eluent pre loaded plate and place the eluent pre loaded plate in position 6 of the extractor.

(Note: The volume of the eluent pre installed on the plate is small. To prevent the liquid from hanging on the sealed aluminum foil film, please make sure the bottom of the plate is facing down and shake it twice with force before tearing off the aluminum foil film, so that all the liquid in each hole slides down to the bottom of the hole.)

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

Step	Hole position	Name	Waiting time (seconds)	Mixing time (seconds)	Magnetic attraction time (seconds)	Mixing speed 1~8	Volume μL	Temperature status	Temperature ℃
1	4	Magnetic transfer	0	20	25	8	700	Close	0
2	1	Combine	0	300	60	7	500	Close	0
3	2	Wash 1	0	60	0	8	700	Close	0
4	1	Combine	0	10	60	8	500	Close	0
5	2	Wash 1	0	0	0	8	700	Close	0
6	1	Combine	0	10	60	8	500	Close	0
7	2	Wash 1	0	180	25	8	700	Close	0
8	3	Wash 1	0	180	25	8	500	Close	0
9	4	Wash 2	0	180	25	8	700	Close	0
10	5	Wash 2	0	180	25	8	500	Close	0
11	6	Elution	300	300	25	5	70	The 6th hole	56
12	4	Abandoning magnetism	0	10	0	8	700	Close	0

3.2.9. Place the 96 hole magnetic rod into the automatic nucleic acid extractor and start the program.

3.2.10. After extraction is complete, the purified nucleic acid is placed in plate 6. Carefully transfer the nucleic acid to a clean 1.5mL 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 attraction parameter of the extractor is the number of magnetic attraction 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: Each component has a clean appearance, no leaks, and no damage; The label should be complete and undamaged, the identification should be clear and complete, and there should be no missing information; Upon visual inspection, there is a light yellow suspension of the settling agent when mixed, which settles into a light yellow color after standing; Visually, RNase A is a colorless and transparent liquid, clear and free of impurities; Visually inspect proteinase K solution as a yellow or light yellow liquid, clear and free of impurities; Visually, when the magnetic bead solution is mixed evenly, it appears as a uniform brownish black color with no precipitate liquid. After standing, the magnetic bead precipitate appears brownish black, and the supernatant is clear and transparent.
2. The A260/A280 ratio of the extracted products in this kit is between 1.7 and 2.1.

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 as having infectious substances

and should not come into contact with the skin and mucous membranes; It is recommended to handle the samples in a biosafety cabinet that can prevent aerosol leakage. The test tubes and suction tips used in the sample preparation area should be placed in containers containing disinfectants and sterilized together with waste before being discarded; Sample handling and processing must comply with relevant regulatory requirements: the Ministry of Health's "General Guidelines for Biosafety in Microbial Biomedical Laboratories" and "Regulations on Medical Waste Management".

3. The components in the reagent kit must be used within their expiration date. Not using the components provided in this 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 once. Specialized instruments and equipment should be used for each stage of experimental operations, and supplies for each zone and stage should not be used interchangeably.
5. Use disposable centrifuge tubes and tips sterilized by high pressure or purchase centrifuge tubes and tips 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 later 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 them with ultraviolet light for 20-30 minutes.