MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Magbead Yeast Plasmind Extraction Kit

Product Number: PLK76

Storage condition

A box is stored at 4~30℃, B box is stored at -20~8℃, and the shelf life is 18 months.

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)

Package	Component	Specifications		
		32 reactions/box	48 reactions/box	64 reactions/box
A box	8F pre packaged reagent board	4 pieces	6 pieces	8 pieces
	16F pre packaged reagent board	2 pieces	3 pieces	4 pieces
	8-link magnetic rod set	4 pieces	6 pieces	8 pieces
	SBT buffer solution	16mL/bottle	24mL/bottle	33mL/bottle
	P1 solution	9mL/bottle	13mL/bottle	18mL/bottle
	P2 solution	9mL/bottle	13mL/bottle	18mL/bottle
	P3 solution	12mL/bottle	18mL/bottle	24mL/bottle
	Eluent	1.2mL/tube	1.2mL/tube	1.2mL/tube
	RNase A	0.18mL/tube	0.26mL/tube	0.36mL/tube
Package	Component	32 reactions/box	48 reactions/box	64 reactions/box
B box	Snail wall lytic enzyme	0.88mL/tube	1.32mL/tube	1.76mL/tube

Pre packaged: 96 reactions/box (product number : PLK76-96)

Package	Component	Specifications	Quantity
A box	SBT buffer solution	47mL/bottle	1 bottle
	P1 solution	26mL/bottle	1 bottle
	P2 solution	26mL/bottle	1 bottle
	P3 solution	36mL/bottle	1 bottle
	Combined board	/	1 piece
	Magnetic bead liquid pre installation plate	96 reactions/block	1 piece
	Washing solution 1 pre installed plate	96 reactions/block	1 piece
	Washing solution 2 pre installed plate	96 reactions/block	2 piece
	Pre loaded eluent plate	96 reactions/block	1 piece
	Eluent	1.2mL/tube	1 tube
	96 hole magnetic rod sleeve	96 reactions/block	1 piece
	RNase A	0.56mL/tube	1 tube
Package	Component	Specifications	Quantity
B box	Snail wall lytic enzyme	2.7mL/tube	1 tube

Large packaging: 50 reactions/box (product number : PLK76-50)

Package	Component	Specifications	Quantity

Package	Component	Specifications	Quantity
A box	SBT buffer solution	25mL/bottle	1 bottle
	P1 solution	14mL/bottle	1 bottle
	P2 solution	14mL/bottle	1 bottle
	P3 solution	19mL/bottle	1 bottle
	Washing solution 1	27mL/bottle	1 bottle
	Washing solution 2	52mL/bottle	1 bottle
	Magnetic bead solution	1.1mL/tube	1 tube
	Eluent	5mL/tube	1 tube
	RNase A	0.28mL/tube	1 tube
B box	Snail wall lytic enzyme	1.4mL/tube	1 tube

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.

Description

This kit uses snail lytic enzyme to digest and remove the thick cell wall of yeast, and is combined with P1 solution, P2 solution, and P3 solution in a unique buffer system to efficiently lyse yeast. Subsequently, the reagent kit uses magnetic beads with unique separation properties to rapidly remove proteins, genomic DNA, and RNA from nucleic acids through binding, washing, and elution steps, allowing plasmid DNA to be released. The purified plasmid DNA can be directly applied to molecular biology experiments such as PCR templates, enzyme digestion, hybridization, etc.

This product can be perfectly matched with an automatic nucleic acid extractor. By using a specially designed magnetic rod to adsorb, transfer, and release magnetic beads, 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 plasmid DNA has high purity without contamination from proteins and other impurities.

Application

This product is suitable for rapid extraction, enrichment, and purification of plasmid DNA from yeast. The processed product is only used for scientific research.

Note

1. Before use, please check whether there is turbidity or precipitation in the P2 solution and P3 solution. If there is turbidity or precipitation, it can be dissolved by heating in a 37℃ water bath for 10 minutes without affecting the effect.
2. Be careful not to directly contact P2 solution and P3 solution, and immediately cover the lid after use.
3. All centrifugation steps were performed using a conventional desktop centrifuge at room temperature.
4. The amount of extracted plasmids is related to factors such as yeast culture concentration and plasmid copy number.
5. Add all RNase A to the P1 solution before use, mix well, and store at 2-8℃.

Sample requirements

1. Applicable sample types: various yeast strains containing plasmids.
2. Sample collection: Take fresh cultured bacterial solution.
3. Sample preservation and transportation: Samples can be used for testing immediately or stored at -20 ± 5 ℃ for testing; Sample transportation is carried out using 0℃ ice pots.

Self provided reagents

β - mercaptoethanol (GC)

Protocol

1. **Manual operation steps (the following operation is applicable to extracting yeast with a concentration not exceeding 5 × 10^7 after overnight cultivation)**

1.1. Cultivate yeast according to standardized procedures. Take 1-5mL of culture medium (<5 × 10^7 yeast cells) and centrifuge in a 2mL centrifuge tube at 12000x g for 1 minute to collect yeast cells. Be careful to discard the culture medium (if there is a large amount of bacterial liquid, multiple centrifugation can be used to collect the bacterial cell sediment in one centrifuge tube).

1.2. Add 470μL SBT buffer, 5μL β - mercaptoethanol (operated in a ventilated environment), and 25μL snail wall lytic enzyme to the sample, resuspend the bacterial cells in a vortex, and incubate at 37℃ for 1-2 hours.

1.3. Centrifuge at 12000x g for 1 minute to collect yeast protoplasts, carefully discard the supernatant.

1.4. Add 255μL of P1 solution (please check if RNase A has been added first), vortex thoroughly to resuspend the precipitate, and let it stand at room temperature for 5 minutes. (At this time, the solution is a turbid light red solution).

Note: If there are incompletely mixed bacterial blocks, it will affect the lysis and result in lower extraction volume and purity.

1.5. Add 250μL of P2 solution to the centrifuge tube and gently flip it up and down 6-8 times to fully lyse the bacterial cells. (After adding P2 solution and mixing well, the solution changes from a turbid light red solution to a clear rose red solution).

Note: Mix gently without vigorous shaking to avoid interrupting genomic DNA and causing genomic DNA fragments to mix in the extracted plasmid. At this point, the bacterial solution should become clear and viscous, and the time used should not exceed 5 minutes to avoid damage to the plasmid. If the liquid has not become clear and transparent, it may be due to excessive bacterial growth and incomplete lysis, and the bacterial volume should be reduced.

1.6. Add 350μL of solution P3 to the centrifuge tube, gently flip it up and down 8-10 times, mix thoroughly, and a flocculent precipitate will appear. (After adding P3 solution and mixing well, the solution turns from rosy red to yellow)

Note: After adding P3 solution, it should be mixed immediately to avoid local precipitation. If the yellow liquid is mixed with rose red, it indicates insufficient reconstitution. Continue mixing until the solution color completely turns yellow.

1.7. After mixing, centrifuge at 12000x g for 10 minutes, and transfer all the supernatant to a new 2 mL centrifuge tube.

1.8. Add 20μL of mixed magnetic bead solution to the centrifuge tube, vortex and mix for 5 minutes, use a magnetic rack to adsorb the magnetic beads, and after 30 seconds, remove the liquid from the tube and remove the centrifuge tube.

1.9. Add 500μL of washing solution 1 to the centrifuge tube, vortex for 1 minute to evenly suspend the magnetic beads again, use a magnetic rack to adsorb the magnetic beads, remove the liquid in the tube after 30 seconds, and remove the centrifuge tube.

1.10. Add 500μL of washing solution 2 to the centrifuge tube, vortex for 1 minute to resuspend the magnetic beads evenly, use a magnetic rack to adsorb the magnetic beads, remove the liquid in the tube after 30 seconds, and remove the centrifuge tube.

1.11. Add 500μL of washing solution 2 to the centrifuge tube, vortex for 1 minute to resuspend the magnetic beads evenly, use a magnetic rack to adsorb the magnetic beads, remove the liquid in the tube after 30 seconds, and remove the centrifuge tube.

1.12. Place the centrifuge tube on a magnetic rack for magnetic bead adsorption, and air dry at room temperature for 5 minutes to remove residual washing solution from the magnetic beads.

Note: Ethanol residue in the washing solution can affect subsequent enzyme reactions (such as enzyme digestion, PCR, etc.) experiments. To ensure that downstream experiments are not affected by residual ethanol, it is necessary to air dry the magnetic beads until the surface is dull, in order to thoroughly dry the residual washing solution on the magnetic beads.

1.13. Remove the centrifuge tube from the magnetic rack, add 30-50μL of eluent, vortex for 5 minutes to resuspend the magnetic beads evenly.

1.14. Place the centrifuge tube on the magnetic rack and adsorb the magnetic beads for 30 seconds. Transfer the liquid to a clean 1.5mL centrifuge tube for later use. The purified plasmid can be used for downstream analysis. If not in a hurry to use, the plasmid solution can be stored in a -20 ℃ refrigerator for later use.

2. Automated operation steps

2.1. Strip pre packaged reagent extraction scheme (note: only the first column of the 8-reaction pre packaged reagent plate is the sampling well).

2.1.1. Sample pre-processing: Same as manual extraction steps 1-7, after completing the pre-processing steps, it is used as a backup sample for testing.

2.1.2. Invert the 96 well reagent plate placed at room temperature three times, centrifuge briefly (or shake by hand) in the 96 well plate centrifuge to avoid liquid hanging, tear off the aluminum foil film on the 96 well reagent plate, and confirm the direction of the reagent plate (magnetic beads in column 2/8).

2.1.3. Add all the test samples mentioned above to the binding well (column 1/7).

2.1.4. Set extraction program:

Step	Hole position	Name	Waiting time (seconds)	Mixing time (seconds)	Magnetic attraction time (seconds)	Mixing speed 1-10	Volume μL	Temperature status	Temperature ℃
1	2	Magnetic transfer	0	20	20	8	500	Close	0
2	1	Combine	0	300	10	7	500	Close	0
3	3	Washing 1	0	60	10	7	500	Close	0
4	4	Washing 2	0	60	10	7	500	Close	0
5	5	Washing 2	0	60	10	7	500	Close	0
6	6	Elution	300	300	10	5	500	Close	0
7	4	Abandoning magnetism	0	10	5	7	500	Close	0

2.1.5. Put 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.

2.1.6. 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 magnetic attraction time, which is 20 seconds per time.

2.2. Plate pre packaged reagent extraction plan: (product number : PLK76-96)

2.2.1. Sample pre-processing: Same as manual extraction steps 1-7, after completing the pre-processing steps, it is used as a backup sample for testing.

2.2.2. 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.

2.2.3. Tear off the sealing aluminum foil film of the binding plate, add all the samples to be tested into the binding plate, and place the binding plate with the added samples in the No.1 position of the extractor.

2.2.4. Tear off the sealing aluminum foil film of the magnetic bead liquid pre installation plate, and place the magnetic bead liquid pre installation plate in position 2 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.

2.2.5. Tear off the sealing aluminum foil film of the pre installed plate of detergent 1, and place the pre installed plate of detergent 1 in position 3 of the extractor.

2.2.6. Tear off the sealing aluminum foil film of the pre installed detergent 2 plate, and place the two pre installed detergent 2 plates in positions 4 and 5 of the extractor.

2.2.7. 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.

2.2.8. Set extraction program:

Step	Hole position	Name	Waiting time (seconds)	Mixing time (seconds)	Magnetic attraction time (seconds)	Mixing speed 1-10	Volume μL	Temperature status	Temperature ℃
1	2	Magnetic transfer	0	20	20	8	500	Close	0
2	1	Combine	0	300	10	7	500	Close	0
3	3	Washing 1	0	60	10	7	600	Close	0
4	4	Washing 2	0	60	10	7	500	Close	0
5	5	Washing 2	0	60	10	7	500	Close	0
6	6	Elution	300	300	10	5	60	Close	0
7	4	Abandoning magnetism	0	10	5	7	500	Close	0

2.2.9. Insert the 96 hole magnetic rod into the automatic nucleic acid extractor and start the program.

2.2.10. After the program runs, the purified nucleic acid is transferred to a clean 1.5mL centrifuge tube for backup in plate 6. 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 magnetic attraction time, which is 20 seconds per time.

Limitations of the product

The efficiency of sample extraction is related to whether the operator strictly follows the instructions. If cross contamination is not properly controlled during sample processing, it may result in low nucleic acid concentration.

Product performance indicators

1. The appearance of each component is clean, leak free, and undamaged; The label should be complete and undamaged, the identification should be clear and complete, and there should be no missing information; Visually, when the magnetic bead solution is mixed evenly, it appears as a black and uniform liquid with no sediment. After standing, the magnetic bead sediment appears black, and the supernatant is clear and transparent; Visually, snail lytic enzyme appears as a brown transparent liquid, clear and free of impurities; Visually, RNase appears as a colorless or pale yellow liquid; Visually, the P1 solution appears as a clear rose red liquid.
2. The A260/A280 ratio of the extracted products in this kit is between 1.7 and 2.1.

Note

For Research Use Only

1. Please read this manual carefully before the experiment.
2. Due to hardware limitations, different models of nucleic acid extraction devices may require different extraction programs. For detailed parameters, please consult our company.
3. 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".
4. 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.
5. 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.
6. Use disposable centrifuge tubes and tips sterilized by high pressure or purchase centrifuge tubes and tips without DNA/RNA enzymes.
7. 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).
8. 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.