Yeast Plasmid Miniprep Kit (Beads)

(BW-MYD1271)

BEIWO This kit is available from yeast which uses a reversible adsorption system of magnetic beads with plasmid DNA, allowing DNA and magnetic beads to bind efficiently while proteins and other contaminants are removed under certain conditions. Nucleic acids are easily eluted with sterile water or an elution buffer. Purified DNA can be used for downstream applications such as enzyme digestion mapping, library screening, sequencing, gene therapy, and gene inoculation.

This kit can purify high quality plasmid DNA in less than 1 hour. This method has been successfully used to isolate and purify plasmids from Saccharomyces cerevisiae as a modified alkaline lysis process in which genomic DNA is normally removed. The kit can be matched with a variety of automated nucleic acid extractors, such as Allsheng Auto-Pure 96A.

Kit Contents

Catalog#	BW-MYD1271-A96-10	BW-MYD1271-A96-11	BW-MYD1271-A96-12
Preps	1 x96	4 x 96	10 x 96
Buffer SE	30 mL	120 mL	300mL
Buffer A1	20mL	80 mL	200 mL
Buffer B1	20mL	80 mL	200 mL
Buffer C1	20mL	80 mL	200 mL
Lyticase solution	10 mL	40 mL	100
RNase A (20mg/ml)	250μL	1mL	2.4mL
Binding Buffer	1	4	10
Buffer KB Buffer	1	4	10
DNA Wash buffer	1*2	4*2	10*2
Elution Buffer	1	4	10
Plasmid-L Bead	1	4	10
Magnetic Rod Sleeve	1	4	10
User Manual	1	1	1

Product storage and stability

This kit can be stored for 12 months from the date of production. Buffer A1 with RNase A added should be stored at 4℃, Lyticase at -20℃, and other reagents and supplies at room temperature (15-25℃).

Key points

- RNase A: 20 mg/mL. Stable storage at room temperature (15-25℃) for one year.
- All RNase A supplied are added to Buffer A1 after instantaneous centrifugation prior to use.
- After use to save Buffer A1 / RNase A at 4 ℃.
- Buffer B1: under room temperature, precipitation, precipitation in 37 ℃ water bath heating to completely dissolved, the solution to clarify. Ensure that the Buffer B1 cap is tightened after use.
- Plasmid - L Beads: needs fully vortex mixing before use. It is recommended to separate according to their own use, avoid repeatedly opening the cover and eddy to reduce the magnetic bead magnetism, magnetic bead debris increase.

Materials to be prepared before the experiment

- isopropyl alcohol
- DTT (DL – Dithiothreitol)

Procedure (magnetic bead method)

1. Fill each well of a 96-well S-Block with 1-1.3 ml of YPD medium containing the appropriate selective agent.
2. Inoculate each well from a single yeast colony or preculture. Incubate the cultures for24-36h at 30°C, with vigorous shaking.
3. Harvest the yeast cells in the block by centrifuging for 5 min at 2100 x g in a centrifuge with a rotor for a 96-well adapter, preferably at 4–10°C. To remove the media, peel off the tape and quickly invert the block over a waste container. Tap the inverted block firmly on a paper towel to remove any remaining droplets of media.
4. Add 50mg glass and 50ul water, vortex for 10 minutes at a maximum speed, let the glass beads settle, transfer the supernatant to a new 1.5ml centrifuge tube, and add **300μL Buffer SE** and 100μl Lyticase solution to reinsert the bacteria. The maximum velocity vortex for 1 minute fully suspended yeast cells. Sufficient suspension of thalli is conducive to high yield. Oscillate at 220rpm at 30°C for 15 minutes, centrifuging for 5 min at 5000 x g, To remove supernatant, and leaving the pellet.

   Note: Be sure to add 20μL DTT (2M) to 1 mL Buffer SE before use. The mixture can be stored at room temperature for 1 month.
5. Add **200 μL Buffer A1 t**o each well (Add **RNase A** to Buffer A1 before use) and completely resuspend yeast pellet by vortexing or pipetting.
6. Add **200μL Buffer B1** to each well, rotate upside down and mix for 4-6 times until clear lysate is obtained. Incubate at room temperature for 4 minutes.

   Note: Avoid violent mixing, or it will cause chromosome DNA break, thus reducing the purity of the plasmid. Tighten the BufferB1 cap after use.
7. Add **200μL BufferC1** to each well, Mix manually for several times until white precipitate floats are formed, centrifuge at room temperature for 10 minutes at 12,000 ×g.
8. Transfer the supernatant to a Binding plate (to avoid precipitation), add one-half volume of **isopropyl alcohol** to the cleared lysate in each well of the Binding Plate.
9. Take six 96-well deep-well plates according to Table 1 below. If the kit is not pre-installed, the following reagents need to be added by yourself.(The total volume of each well must not exceed 1,000 μL,)

Table 1 plate set-up

96-well plate No.	Board position	Sample / reagent	Kit description	Note
Heat	1	lysate supernatant	Added by user	Try to take the clear lysate, and then add isopropyl alcohol in turn to get the mixture.
		isopropyl alcohol	Added by user	
Beads	3	Plasmid-L Beads	The reagent has been added, no need for user to add	/
Wash 1	4	Buffer KB	The reagent has been added, no need for user to add	/
Wash 2	5	DNA Wash Buffer	The reagent has been added, no need for user to add	/
Wash 3	6	DNA Wash Buffer	The reagent has been added, no need for user to add	/
Elution	8	Elution Buffer	The reagent has been added, no need for user to add	Elution volume can be adjusted according to specific requirements, at least 60ul.

10. Start the instrument, place new clean magnetic bar sleeve in the instrument, and put 96-well plates into the corresponding position in the instrument, corresponding to the magnetic bar sleeve. Use the program (table 2).
11. Collect products after the program is completed. Take out 96-well plate, and store at -20℃ or -80℃.

Table 2. Extraction procedures

step	name	plate position	Mix time (min)	Magnetize time (s)	Wait time (min)	Vol (μL)	Mix speed (1-10)	Tm (℃)	Mix position (0-100%)	Mix range (1-100%)	Magnetize position (0-100%)	Magnetize speed (1-10)
1	Load	3	-	-	-	-	-	-	-	-	-	-
2	Beads	3	0	80	0	100	-	OFF	1	2	1	20
3	Banding	1	5	80	0	1000	3	OFF	3	2	1	15
4	Wash1	4	0.5	80	0	500	5	OFF	3	2	1	10
5	Wash2	5	0.5	80	0	600	5	OFF	3	2	1	10
6	Wash3	6	0.5	80	2	600	5	OFF	3	2	1	10
7	Elute	8	5	80	0	100	3	65	4	2	1	30
8	Drop	3	0.2			750	6					
9	Unload	3	-	-	-	-	-	-	-	-	-	-

Note: Set to heat up and then action, cooling action synchronization; magnetize mode: magnetize in 4 stages; drying position: upper part of the kit.

Trouble Shooting Guide

Problems	Possible Reasons	Suggested Improvements
Low yield	Poor cell lysis.	Resuspend pellet throughly by vortexing and pipetting prior to adding Buffer B1.
		Make fresh Buffer B1 if the cap had not been closed tightly. (Buffer B1: 0.2 M NaOH and 1% SDS).
Low yield	Low copy number plasmid.	Increase culture volume and the volume of Buffer A1,B1,N1 as instructed on page 9.
Genomic DNA contamination	Over-time incubation after adding Buffer B1.	Do not vortex or mix aggressively after adding Buffer B1. Do not incubate more than 5 minutes after adding Buffer B1.
RNA contamination	RNase A not added to Buffer A1.	Add RNase A to Buffer A1.
Plasmid DNA floats out of wells while running in agarose gel	Ethanol traces were not completely removed from column.	Make sure that no ethanol residue remains in the silicon membrane before elute the plasmid DNA. Re-centrifuge or vacuum again if necessary.

Limited Use and Warranty

This product is intended for in vitro research use only. Not for use in human.

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind expressed or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product. For technical support or learn more product information, please contact us or visit our website.

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