EasyPure® 1-Tube Universal EndoFree Plasmid MaxiPrep Kit (for 100-500 ml)

Please read the manual carefully before use.

Cat. No. EM152

Version No. Version 1.0

Storage: at 15℃-30℃ for one year.

Description

This product is universal plasmid maxiprep kit. It uses a modified lysis system to lyse *E.coli* cells to efficiently release plasmid DNA, followed by the binding of the DNA onto the silica membrane spin column. Lysis and neutralization of 100-500 ml *E.coli* liquid cultured in LB medium can be completed in a single centrifuge tube, the volume of the supernatant is less than 50 ml. The solution contains indicators that can indicate whether the lysis and neutralization are complete through the change of color, so as to visualize the operation.The purified DNA is suitable for restriction enzyme digestion, ligation, transformation, sequencing, transfection, etc.

Features

- 1-tube type: Lysis and neutralization of 100-500 ml bacterial liquid can be completed in a single 50 ml centrifuge tube, without the need to prepare large-volume consumables.
- Visualization: Solution LB II (blue) indicates whether lysis and neutralization are complete through the change of color, ensuring the quality of plasmid extraction.
- Fast: The universal system is compatible with extraction of 100-500 ml bacterial liquid. Small volume system combined with large volume spin column greatly reduces the number of centrifugation and saves operation time.
- High yield: Up to 5 mg nucleic acid load in the purification column.

Self-prepared

Isopropanol (analytically pure), absolute ethanol (analytically pure), high-speed centrifuge, thermostatic water bath, 50 ml centrifuge tubes.

Kit Contents

Component	EM152-01 (10 rxns)
Resuspension Buffer II (RB II)	140 ml
Lysis Buffer II (LB II, Blue)	70 ml×2
Neutralization Buffer II (NB II)	140 ml
Activation Buffer II (AB II)	55 ml
Wash Buffer II (WB II)	25 ml
Elution Buffer (EB)	31 ml
RNase A (10 mg/ml)	1.4 ml
Maxi-Plasmid Spin Column with Collection Tube	10 each
Push Filter	10 each
50 ml Collection Tube	10 each

Procedures

Prior to use, add RNaseA to RB II, store at 2-8°C; add 100 ml of absolute ethanol to WB II.

1. Incubate the bacterial liquid in LB Media for overnight (14-16 hours). Harvest 100-500 ml cultured bacterial cells (OD_{600}≤3.0) by centrifuging at 10,000 × g for 3 minutes and discarding the supernatant. (For low-copy plasmids or large plasmids (>15 kb), the recommended minimum volume of bacterial culture is 200 ml. Maximum sample size is shown in the table below.)

Maximum sample size	Bacterial sludge	Bacterial liquid				
	Mass	OD_{600}*V (Volume, ml)	OD_{600}=2	OD_{600}=3	OD_{600}=4	OD_{600}=5
	2.0 g	1500	750 ml	500 ml	375 ml	300 ml

2. Column activation: add 5 ml of column activation solution AB II to the center of Maxi-Plasmid Spin Column with Collection Tube, stay still for 2 minutes at room temperature. Centrifuge at 8,000 × g for 1 minute, discard the effluent and set aside. (Activated spin columns should be used as soon as possible within 1 hour)
3. Add 12 ml of colorless solution RB II (containing RNase A). Mix thoroughly by vortexing. And there should be no small bacterial masses. Transfer the resuspended bacteria to a 50 ml centrifuge tube for subsequent operations.
4. Add 12 ml of blue solution LB II, gently flip up and down and mix 15-20 times until a uniform blue-green transparent solution is formed (For large volume of bacterial cells, the mixing times can be increased appropriately), stay still for 5 minutes at room temperature.
5. Add 12 ml of solution NB II to the step 4 lysate, mix by inverting several times until the color of the solution completely changes from blue-green to colorless and dispersed clump (egg flower-shaped) appears, indicating complete neutralization. Stay still for 2 minutes at room temperature.
6. Centrifuge at 10,000 × g for 15 minutes (More bacteria can appropriately extend the centrifugation time). Carefully avoid precipitation, pour the supernatant into the Push Filter and push into a new 50 ml centrifuge tube (self-prepared). (Slowly pull up the filter rod to prevent the filter from loosening and affecting the filter effect)
7. Add 0.3 times the volume of isopropanol and mix by inverting the tube. Transfer the liquid to the spin column in 3 times. Centrifuge at 8,000 × g each time for 1 minute. Discard the effluent. (It is recommended that the volume of a single column should not exceed 17 ml, and the height of the liquid should not exceed the "△ MAX△" line)
8. Add 5 ml of solution WB II. Centrifuge at 8,000 × g for 1 minute. Discard the effluent.
9. Repeat step 8 once.
10. Centrifuge at 8,000 × g for 3 minutes to thoroughly remove residual WB II. Place the spin column in a new 50 ml Collection Tube. Leave the column open for 5-10 minutes at room temperature to allow the ethanol to evaporate clean.
11. Add 1-3 ml of EB or deionized water (7.0<pH<8.5) to the center of the spin column and stay still for 5 minutes at room temperature. (EB or deionized water is better used after preheating in a water bath at 60-70°C)
12. Centrifuge the column at 8,000 × g for 2 minutes to elute DNA. (Approximate 60%-70% recovery rate for first elution. To increase plasmid DNA recovery, repeat this step after adding the eluate back to the center of the spin column and keeping it still for 5 minutes)
13. Isolated plasmid DNA can be stored at -20°C.

Optional (to futher concentrate DNA)

1. Transfer eluate to a microcentrifuge tube, add 1/10 volume of NB II and 7/10 volume of isopropanol (room temperature), mix well and stay still for 5 minutes at room temperature.
2. Centrifuge at 12,000 × g for 10 minutes at room temperature and carefully discard the supernatant (If the precipitate is not tightly adhered to the wall, the centrifugation time can be extended).
3. Add 1 ml of 70% ethanol (room temperature), vortex for 10 seconds, centrifuge at 12,000 × g for 10 minutes at room temperature and discard the supernatant. Then brief centrifugation and aspirate the residual liquid.
4. Air-dry the pellet for 5-10 minutes. Add appropriate volume of EB to dissolve the pellet.

Notes

• After adding LB II and NB II, the operation should be gentle, vigorous mixing will lead to genome contamination.
• Add the whole volume of RNase A (supplied with this kit) into RB II, mix thoroughly and store at 2-8°C.

- Prior to use, check whether LB II or AB II is cloudy or not, if it is cloudy, heat it in 37℃ water bath to completely dissolve it. Tighten the cap immediately after use to avoid pH change.
- Strictly control cell culture input volume. When extracting bacterial liquid cultured in LB Media, the OD_{600}*V value exceeds 1500 or the mass of bacterial sludge exceeds 2.0 g, which will lead to incomplete lysis and insufficient endotoxin removal, affecting the yield and purity of plasmid DNA. When extracting bacterial liquid cultured in rich culture media such as TB Media or 2×YT Media, the amount of bacterial liquid should be appropriately reduced.
- The elution volume should not be less than 1 ml, too small the elution volume will affect the elution efficiency.
- It is recommended to detect the quality of the extracted plasmid DNA (presence or absence of RNA, genomic DNA residue, and proportion of supercoiled plasmid) by agarose gel electrophoresis. Residual RNA or genomic DNA will cause the plasmid concentration to be seriously inflated, which will directly affect the quantitative accuracy and the success rate of downstream experiments.

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