ab288094 – Express Plasmid Midiprep Kit

For the fast and efficient purification of plasmid DNA from 50-80 mL of E. coli culture.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab288094

Storage and Stability

All the reagents are shipped at room temperature. Except the Buffer A1 (once RNase A is added), which is stored at 4°C, all other components are stored at room temperature. the guaranteed shelf life is 12 months from the date of purchase. **Do not freeze.**

Materials Supplied

Item	Quantity	Storage Condition
ezBind Columns	2	RT
Syringe Filters	2	RT
Collection Tubes	4	RT
Plastic Wrench	1	RT
Buffer A1	12 mL	-4°C
Buffer B1	12 mL	RT
Buffer C1	15 mL	RT
DNA Washing Buffer*	5 mL	RT
RNase A	50 µL	RT
Elution Buffer	5 mL	RT

*Add 20mL 100% ethanol to each DNA Wash Buffer before use

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:
100% ethanol

- High speed centrifuge
- 50mL high speed centrifuge tubes
- 50mL conical tubes
- Isopropanol if precipitating the plasmid DNA

Reagent Preparation and Storage Conditions

- RNase A is stable for more than half a year when stored at room temperature.
- Spin down RNase A vial briefly. Add the RNase A solution to Buffer A1 and mix well before use.
- Buffer B1 precipitates below room temperature. It is critical to warm up the buffer at 50°C to dissolve the precipitates before use.
- Buffer C1 may form precipitates below 10°C, warm up at 37°C to dissolve the precipitates before use.
- Keep the cap tightly closed for Buffer B1 after use.
- Make sure the availability of centrifuge (13,000 rpm). Especially, after mixing the lysate with ethanol, the sample needs to be processed immediately by centrifugation.
- Carry out all centrifugations at room temperature.

Express Plasmid Midiprep Protocol

The protocols are optimized for high copy number plasmid purification. For low copy number plasmids, both the culture volume and the buffer volume need to be scaled up 2 times.

1. Inoculate 50-80 mL LB containing appropriate antibiotic with 100 µl fresh starter culture. Grow at 37oC for 14-16 hr with vigorous shaking.

Note: The best way to prepare a starter culture: Inoculate a single colony from a freshly grown selective plate into 1 ml LB medium containing the appropriate antibiotic and grow at 37°C for 6-8 hr with vigorous shaking (~250 rpm).

Warning: Do not use more than 100 ml culture. Need to scale up buffers if processing more than 100 mL culture.

2. Harvest the bacterial by centrifugation at 5000g for 10 min at room temperature. Pour off the supernatant and blot the inverted tube on paper towels to remove residual medium.

Note: Complete removal of residue medium is critical for bacteria lysis in the next step.

3. Add 5 mL Buffer A1 and completely resuspend bacterial pellet by vortexing or pipetting. Ensure that RNase A has been added into Buffer A1 before use. Note: Complete resuspension is critical for optimal yield.
4. Add 5 mL Buffer B1, mix thoroughly by inverting 10 times with gentle shaking. Incubate for 5 -10 min to obtain a slightly clear lysate. Complete lysis is critical for DNA yield. The mixture of completely lysed bacteria looks transparent.

Note: Buffer B1 forms precipitation below room temperature, if solution becomes cloudy, warm up at 37oC to dissolve before use.

5. Add 2.5 ml Buffer C1, mix completely by inverting the tube 10 times and shaking for 5 times. It is critical to mix the solution well, if the mixture still appears conglobated, brownish or viscous; more mix is required to completely neutralize the solution.
6. Two options for clearing the lysates: High speed centrifuge: Transfer the lysate to a high speed centrifuge tube and centrifuge at 13,000 rpm (14,000g-18,000g) for 15 min at room temperature! Transfer the cleared lysate to a 50 ml conical tube. Add 3.0 ml Buffer C1 and mix well. Go to step 7.

ezFilter syringe: Incubate at room temperature for 10 min. Add 3.0 ml Buffer C1 and mix well. Pour the lysate into the barrel of the filter syringe and set the syringe in a 50 mL conical tube. The white precipitates should float to the top. Gently insert the plunger to expel the cleared lysate to the tube, stop when feel strong resistance, some of the lysate may remain in the flocculent precipitate.

Note: To avoid clog of the syringe: Use less than 100 mL of overnight culture and mix the lysate well after adding Buffer C1. Alternatively, transfer the lysate to another syringe filter.

7. Add 5 mL absolute ethanol (96-100%) to the cleared lysate. Mix well by sharp shaking for 5 times.
8. Add the lysate/ethanol mixture into a DNA column set in a 50 mL conical tube. Use the plunger to expel the lysate through the column.
9. Gently pull the plunger out, add 10 mL DNA Wash Buffer, expel the Buffer out with the plunger. Expel the liquid completely by push and pull of the plunger several times.
10. Use the plastic wrench (Provided) to detach the end component from the midiprep column and insert it into a 2.0 ml Eppendorf tube.
11. Centrifuge the column at 13,000-15,000 rpm (Max speed) for 1 min. Decant the flow through and put the column back to the tube. Spin the column at max speed for 2 min.
12. Add 400 µL Elution Buffer (Prewarm the Elution Buffer 60°C increases the yield) to the center of the column and incubate for 1 min at room temperature. Elute the DNA by centrifugation at 13,000 rpm for 1 min.
13. Transfer the column to a new 1.5 mL tube and add 0.2 mL Elution Buffer to the column for a second elution. The first elution yields 60-70% of the DNA while the second elution yields another 20-30% of the DNA bound to the column.

Note: The DNA is ready for downstream applications such as cloning, RFLP, sequencing and transfection of HEK293 cells.

Note: It's highly recommended to remove the endotoxin if the DNA is used for endotoxin-sensitive cell lines, primary cultured cells or microinjection.

Technical Support

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