ab288092 – Express Plasmid Maxiprep Kit

The Maxiprep Kit is designed for fast and efficient purification of plasmid DNA from 150-200 mL of *E. coli* culture.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
https://www.abcam.com/ab288092

Storage and Stability

The All the reagents are shipped at room temperature (RT). RNase A should be stored at 4°C. Buffer A1 (once RNase A is added), is also stored at 4°C. All other components are stored at RT. The guaranteed shelf life is 18 months from the date of purchase. DO NOT FREEZE!

Materials Supplied

Components	2 preparations	10 preparations	25 preparations	Storage
ezBind Columns	2	10	25	RT
Filter Syringe	2	10	25	RT
2.0 mL Microfuge Tube	4	20	50	RT
Plastic Wrench	1	1	1	RT
Buffer A1	22 mL	110 mL	270 mL	RT
Buffer B1	22 mL	110 mL	270 mL	RT
Buffer C1	27 mL	135 mL	340 mL	RT
DNA Washing Buffer	15 mL	45 mL	2 x 45 mL	RT
RNase A (20 mg/mL)	100 µL	420 µL	900 µL	+4°C
Elution Buffer	5 mL	15 mL	50 mL	+4°C

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

ΔNote: *Add 48 ml (2 preparations) or 200 mL (10/25 preparations) of 100% ethanol to each DNA Wash Buffer bottle before use.*

RNase A: Spin down RNase A vial briefly. Add the RNase A solution to Buffer A1 and mix well before use.

- Buffer B1 precipitates below RT. It is critical to warm up the buffer at 50°C to dissolve the precipitates before use.
- Keep the cap tightly closed for Buffer B1 after use.
- Buffer C1 may form precipitates below 10°C, warm up at 37°C to dissolve the precipitates before use.
- After mixing the lysate with ethanol, the sample needs to be processed immediately by centrifugation (13,000 rpm).
- Carry out all centrifugations at RT

Assay Procedure

- The protocol is optimized for high copy number plasmid purification.
- For low copy number plasmids, both the culture volume and the buffer volume need to be doubled.

1. Inoculate 150-200 mL LB containing appropriate antibiotic with 100 µL fresh starter culture. Grow at 37°C for 14-16 h with vigorous shaking.

   Δ Note: *The best method 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 h with vigorous shaking (~250 rpm). Warning: Do not use more than 200 ml culture. You will need to scale up buffers if processing more than 200 mL culture.*

2. Harvest the bacteria by centrifugation at 5,000 g for 10 min at room temperature. Pour off the supernatant and blot the inverted tube on paper towels to remove residual medium.

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

3. Add 10 mL Buffer A1 and completely resuspend bacterial pellet by vortexing or pipetting. Ensure that RNase A has been added into Buffer A1 before use.

   Δ Note: *Complete resuspension is critical for optimal yield.*

4. Add 10 mL Buffer B1, mix thoroughly by inverting 10 times with gentle shaking. Incubate for 5 min to obtain a slightly clear lysate. Complete lysis is critical for DNA yield. The mixture of completely lysed bacteria looks transparent.

   Δ Note: *Buffer B1 forms precipitation below room temperature, if the solution becomes cloudy, warm up at 37°C to dissolve before use.*

5. Add 4 mL Buffer C1, mix completely by inverting 10-15 times. It is critical to mix the solution well if the mixture still appears conglobated, brownish or viscous; more mixing is required to completely neutralize the solution.

6. Two options for clearing the lysates:
   a. High speed centrifuge: Transfer the lysate to a high-speed centrifuge tube and centrifuge at 13,000 rpm for 15 min at room temperature. Transfer the cleared lysate to a 50 ml conical tube. Add 8 mL Buffer C1 and mix well. Go to step 7.
   b. ezFilter syringe: Incubate the lysate at room temperature for 8 min. Add 8 mL Buffer C1 and mix well. Pour the lysate into the barrel of the filter syringe. Hold the syringe for 30 seconds over a clean 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 resistance is felt. Some of the lysate may remain in the flocculent precipitate.

Δ Note: *To avoid clogging of the syringe: Use less than 200 mL of overnight culture; Spin the lysate at 5,000 rpm for 5 min and transfer the relatively clear lysate to the syringe filter barrier.*

7. Add 10 mL absolute ethanol (96-100%) to the cleared lysate. Mix well by sharp shaking 5 times. Proceed with plunger protocol or vacuum manifold protocol (see below).

8. Pull out the plunger from the DNA column and set the column in a 50 mL conical tube. Add the lysate/ethanol mixture into a DNA column. Using the plunger, gently expel the lysate through the column to the conical tube.

9. Use the plastic wrench (Provided) to detach the end component from the maxi column. Gently pull the plunger out. Use the wrench to tightly screw the end component back onto the maxi column. Add 15 mL DNA Wash Buffer and expel the Buffer out with the plunger.

10. Use the plastic wrench to detach the end component from the column and insert it into a collection tube (provided).

11. Centrifuge the column at 13,000-15,000 rpm (Max speed) for 2 min. Decant the flow through and put the column back into the collection tube. Spin the column at max speed for 1 min. Transfer the column to a new collection tube.

12. Add 0.4 mL Elution Buffer to the center of the column and incubate for 1 min at RT. Elute the DNA by centrifugation at 13,000 rpm for 1 min.

13. Transfer the column to a new collection tube and add 0.4 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.

Δ Note: *If high DNA concentration is desired, add the eluted DNA (first elution) back to the DNA column and spin at 13,000 rpm for 1 min. The DNA is ready for downstream applications such as cloning, RFLP, sequencing and transfection of HEK293 cells. It's highly recommended to remove the endotoxin if the DNA is used for endotoxin-sensitive cell lines, primary cultured cells or microinjection.*

Vacuum manifold method (From step 7):

1. Pull out the plunger of the DNA column and fix the column to the manifold with the vacuum off. Transfer the lysate/ethanol mix to the column and turn on the vacuum until all the sample has passed through the column.
2. Add 10 mL DNA Wash Buffer to the column and allow the liquid to pass through the column. Continue vacuum for 1 min. Turn off the vacuum and pull the column out from the manifold.
3. Use the plastic wrench to detach the end component from the midiprep column and insert it into a 1.5 ml eppendorf tube.
4. Spin the column at 13,000-15,000 rpm (Max speed) for 2 min. Decant the flow through and put the column back into the tube. Spin the column at max speed for 2 min. Transfer the contents to a new eppendorf tube.
5. 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.

Technical Support

For all technical or commercial enquiries please go to:

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