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Plasmid Miniprep Kit I

(Cat# K1312-50, -250; Store at multiple temperatures)

I. Introduction:

BioVision's Plasmid Miniprep kit is designed for fast and efficient purification of plasmid DNA from 1-4 mL of E. coli cultures in less than 20 min. The mini column has a plasmid DNA binding capacity of 50 µg. The yield from 1 mL culture is typically around 8-12 µg. However, 10-20 µg of high copy number plasmid DNA can be also isolated from 1-2 mL culture. The purified DNA is ready for downstream applications such as restriction digestions, PCR, and automated sequencing. The key to the kit is the proprietary DNA binding systems that allow the high efficient reversible binding of DNA to the mini column while proteins and other impurities are removed by wash buffer. Nucleic acids are then eluted with sterile water or elution buffer.

II. Sample Type:

For fast and efficient purification of plasmid DNA from 1-4 mL of *E. coli* culture.

III. Kit Contents:

	K1312-50	K1312-250	
Components	**50 preparations**	**250 preparations**	**Part Number**
ezBind Columns	50	250	K1312-XX-1
Buffer A1	15 mL	65 mL	K1312-XX-2
Buffer B1	15 mL	65 mL	K1312-XX-3
Buffer N1	20 mL	90 mL	K1312-XX-4
Buffer KB	15 mL	70 mL	K1312-XX-5
DNA Wash Buffer*	12 mL	50 mL	K1312-XX-6
Elution Buffer	10 mL	30 mL	K1312-XX-7
RNase A (20 mg/mL)	50 µL	210 µL	K1312-XX-8

***Add 48 mL (K1312-50) or 200 mL (K1312-250) of 96-100% ethanol to each DNA Wash Buffer bottle before use**

IV. User Supplied Reagents and Equipment:

- High speed microcentrifuge or Vacuum manifold.
- 96-100% ethanol
- 1.5 mL microcentrifuge tubes

V. Shipment and Storage:

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!

VI. Reagent Preparation and Storage Conditions:

- RNase A: It 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. Store at 4°C.
- Add 48 mL (K1312-50) or 200 mL (K1312-250) of 96-100% ethanol to each DNA Wash Buffer bottle 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.
- Ensure the availability of centrifuge capable of 13,000 rpm.
- Carry out all centrifugations at room temperature.

VII. Plasmid Miniprep Spin Purification 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 **1-3 mL LB** containing appropriate antibiotic with a fresh colony from a freshly streaked selective plate. Incubate at 37°C for 14-16 hr with vigorous shaking. *Note: Prolonged incubation (> 16 hr) is not recommended since the E.coli starts to lyse and the plasmid yields may be reduced. Note: Do not grow the culture directly from the glycerol stock. Note: This protocol is optimized for E. coli strain cultured in LB medium. When using TB or 2X YT medium, special care needs to be taken to ensure the cell density doesn't exceed 3.0 (OD600). Buffers need to be scaled up proportionally if more volume of cultures are being processed.*
2. Harvest the bacterial culture by centrifugation for 1 min at 10,000 rpm. Pour off the supernatant and blot the inverted tube on a paper towel to remove residue medium. Remove the residue medium completely. *Note: Residue medium will cause, poor cell lysis and thus lower DNA yield or loose pellet after centrifugation in step 6.*
3. Add **250 µL Buffer *A1 (*Add RNase A to Buffer A1 before use)** and completely resuspend bacterial pellet by vortexing or pipetting *Note: Complete resuspension is critical for bacterial lysis and lysate neutralization*.
4. Add **250 µL Buffer B1**, mix gently by inverting the tube 10 times (do not vortex), and incubate at room temperature for 5 min. *Note: Do not incubate for more than 5 min. Note: Buffer B1 precipitates (cloudy look) below room temperature. Warm up Buffer B1 at 50°C to dissolve precipitation before use.*
5. Add **350 µL Buffer N1**, mix completely by inverting/shaking the vial for 5 times and sharp hand shaking for 2 times. *Note: Incubating the lysate in ice for 1 min will improve the yield. Note: 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. Centrifuge the lysate at 13,000 rpm for 10 min at room temperature. *Note: If the lysate doesn't appear clean, reverse the tube angle, centrifuge for 5 more min and then transfer the clear lysate to DNA column.*
7. Carefully transfer the clear lysate into a DNA column with a collection tube, avoid the precipitations, spin at 13,000 rpm for 1 min, discard the flow through and put the column back to the collection tube.

8. *Optional: Add **250 µL Buffer KB** into the spin column, centrifuge at 13,000 rpm for 30 sec. Remove the spin column from the tube and discard the flow-through. Put the column back to the collection tube. Note: Buffer KB is recommended for endA+ strains such as HB101, JM101, TG1 or their derived strains. It is not necessary for isolating DNA from endA- strains such as Top 10 and DH5a.*
9. Add **600 µL DNA Wash Buffer** (Add ethanol to DNA wash buffer before use) into the spin column, centrifuge at 13,000 rpm for 1 min at RT. Remove the spin column from the tube and discard the flow through. Repeat step "9" to improve the recovery.
10. Reinsert the spin column, with the lid open, into the collection tube and centrifuge for 1-2 min at 15,000 rpm. *Note: Residual ethanol can be removed more efficiently with the column lid open. It is critical to remove residual ethanol completely.*
11. Carefully transfer the spin column into a sterile 1.5 mL tube and add **50-100 µL** (> 50 µL) Sterile ddH_20 or Elution Buffer into the center of the column and let it stand for 2 min. Elute the DNA by centrifugation at 13,000 rpm for 1 min. Reload the eluate into the column and elute again. *Note: If ddH_2O is applied, please make sure the pH is no less than 7.0 (7.0-8.5 is preferred). NaOH could be used to adjust the pH of ddH_2O. Note: It's highly recommended to remove the endotoxin if the DNA is used for endotoxin-sensitive cell lines, primary cultured cells or microinjection.*

The DNA concentration can be calculated as follows, Concentration (µg/mL) = $OD_{260\ nm}$ X 50 X dilution factor.

VIII. Related Products:

Product Name	Catalog Number
Plasmid Miniprep Kit I	K1312-50, -250
Plasmid Miniprep Kit II	K1313-50, -250
Plasmid Midi Kit I	K1314-2, -10, -25
Plasmid Midi Kit II	K1315-2, -10, -25
Plasmid ezFilter Midi Kit I, Centrifuge	K1316-2, -10, -25
Plasmid ezFilter Midi Kit II, Centrifuge	K1317-2, -10, -25
Plasmid ezFilter Maxi Kit	K1319-2, -10, -25
Plasmid ezFilter Mega3 Kit	K1320-1, -2, -10
Plasmid ezFilter Mega6 Kit	K1321-1, -2, -10
Plasmid ezFilter Mega10 Kit	K1322-1, -2, -10
Express Plasmid Midiprep Kit (25 min)	K1323-2, -10, -25
Express Plasmid Maxiprep Kit (25 min)	K1324-2, -10, -25
96-well Plasmid ezFilter Mini Kit	K1325-100 (1 Pack)
96-well Plasmid ezFilter Mini Kit	K1325-100 (4 Packs)

IX. General Troubleshooting Guide:

Problems	Possible Reasons	Solutions
Low Yield	• Poor Cell lysis. • Bacterial culture. overgrown or not fresh. • Low copy number plasmid.	• Resuspend pellet thoroughly by votexing and pipetting prior to adding Buffer B1. • Make fresh Buffer B1 if the cap had not been closed tightly. (Buffer B1: 0.2M NaOH and 1%SDS). • Grow bacterial 12-16 hr. Spin down cultures and store the pellet at -20 °C if the culture is not purified the same day. Do not store culture at 4°C overnight. • Increase culture volume and the volume of Buffer A1, B1, N1 as instructed.
No DNA	• Plasmid lost in Host *E. coli.*	• Prepare fresh culture.
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 min 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, DNA doesn't freeze or smell of ethanol	• Ethanol traces were not completely removed from the column.	• Make sure that no ethanol residue remains in the silicon membrane before elute the plasmid DNA. Re-centrifuge or vacuum again if necessary.

FOR RESEARCH USE ONLY! Not to be used on humans.