BioVision
BioVision Incorporated

Plasmid Midiprep Kit I

(Cat# K1314-2, -10, -25; Store at RT)

I. Introduction:
BioVision's Plasmid Midiprep Kit I is designed for fast and efficient purification of plasmid DNA from 15-50 mL of *E. coli* cultures in less than 60 min. The midi column has a plasmid DNA binding capacity of 250 µg. 100-200 µg of high copy number plasmid DNA can be isolated from 50 mL culture. The yield from 50 mL culture is typically around 150-250 µg. The purified DNA is ready for downstream applications such as transfection, RFLP, DNA amplification, and automated sequencing. Unlike other kits in the markets, our patented plasmid purification kit has no chaotropic salts in the buffers, the purified DNA is guanidine/anion exchange resin residues free which enable the high performance of downstream applications.

II. Sample Type: For fast and efficient purification of plasmid DNA from 15-50 mL of *E. coli* culture.

III. Kit Contents:

Components	K1314-2 2 Preparations	K1314-10 10 Preparations	K1314-25 25 Preparations	Part Number	Storage Temperature
Midi Columns	2	10	25	K1314-XX-1	RT
Buffer A1	6 mL	30 mL	70 mL	K1314-XX-2	RT
Buffer B1	6 mL	30 mL	70 mL	K1314-XX-3	RT
Buffer C1*	7 mL	35 mL	90 mL	K1314-XX-4	RT
DNA Wash Buffer	5 mL	50 mL	50 mL	K1314-XX-5	RT
RNase A (20 mg/mL)	30 µL	110 µL	260 µL	K1314-XX-6	RT
Elution Buffer	2 mL	10 mL	30 mL	K1314-XX-7	RT

Buffer C1*: It contains acetic acid, wear gloves and protective eyewear while handling. Add 20 ml (K1314-2) and 200 ml (K1314-10 and K1314-25) 100% ethanol to each DNA Wash Buffer.

IV. User Supplied Reagents and Equipment:
- 70% ethanol and 100% ethanol
- High speed centrifuge
- 30 mL high speed centrifuge tubes
- 15 mL; 1.5 mL tubes
- Isopropanol if precipitating the plasmid DNA

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: 20 mg/mL. 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.
- 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.
- Make sure the availability of centrifuge, especially, after mixing the lysate with ethanol, the sample needs to be processed immediately either by centrifugation.
- Carry out all centrifugations at room temperature.

VII. Plasmid Midiprep Spin 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 **15-50 mL** LB containing appropriate antibiotic with 50 µL fresh starter culture. Incubate at 37°C 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 8 hr with vigorous shaking (~250 rpm) and then use the culture as starter culture. Note: Do not use more than 50 mL culture or cell mass greater than 150. The buffer volume needs to be scaled up if processing over 100 mL of culture. Note: Do not use a starter culture that has been stored at 4°C. Note: Do not grow starter culture directly from glycerol stock.*
2. Harvest the bacterial by centrifugation at 5,000g for 10 min at room temperature. Pour off the supernatant and blot the inverted tube on paper towels to remove residual medium.
3. Add **2.5 mL Buffer A1** (Add RNase A to Buffer A1 before use) and completely resuspend bacterial pellet by vortexing or pipetting (Complete resuspension is critical for optimal yields).
4. Add **2.5 mL Buffer B1**, mix gently but thoroughly by inverting 5 times and incubate for 5 min to obtain a slightly clear lysate. *Note: Do not incubate longer than 5 min. Over-incubating causes genomic DNA contamination and plasmid damage.*
5. Add **1.0 mL** Buffer C1, mix immediately by inverting 10 times and sharp hand shaking for 3 times. *Note: 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 for 10 min at room temperature. Transfer the cleared lysate to a 15 mL conical tube. Add 2.5 mL Buffer C1.
   ezFilter Syringe (EZfilter Kit): Incubate the lysate at RT for 10 min. Add **2 mL of Buffer C1** and mix well. Pour the lysate into the barrel of the filter syringe. Hold the syringe for 20 sec over a clean 10 mL conical tube. The white precipitates should float on the top. Gently insert the plunger to expel the cleared lysate to the tube, stop when feel 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.* Carefully transfer the clear supernatant into a 15 mL tube (avoid the floating precipitates).
7. Add **3.0 mL 100% ethanol**. Mix immediately by sharp hand-shaking. The mixture of ethanol/lysate needs to be centrifuged through to the DNA column immediately.

8. Immediately transfer **6.0 mL the lysate/ethanol mix** into a DNA column with a 15 mL collection tube. Centrifuge at 5000g for 2 min. Remove the column from the tube and discard the flow-through liquid. Reinsert the column to the collection tube.
9. Add **4.0 mL 70% ethanol** into the column, centrifuge at 5000g for 1 min. Remove the column from the tube and discard the flow through. Reinsert the column into the collection tube. Repeat step "10".
10. Add **4.0 mL 70% ethanol** into the column, centrifuge at 5000g for 1 min. Remove the column from the tube and discard the flow through. Reinsert the column into the collection tube.
11. Centrifuge the column, with the lid open, at 5000g for 10 min to remove the ethanol residues. *Note: Residual ethanol can be removed more efficiently with the column lid open. High centrifuge speed is suggested to remove the ethanol. It is critical to remove residual ethanol completely.* Carefully transfer the column into a sterile clean 15 mL tube
12. **Add 0.5 mL Elution Buffer or sterile ddH$_2$O** to the center of the column and incubate for 1 min at room temperature. Elute the DNA by centrifugation at 5000g for 3min.
13. For higher yield, reload the eluate in the 15 mL tube to the column and incubate for 1 min. Elute the DNA again by centrifugation at 5000g for 5 min. *Note: If ddH$_2$O 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$_2$O. Note: The DNA is ready for downstream applications such as cloning/subcloning, RFLP, Library screening, in vitro translation, sequencing, transfection of robust HEK293 cells. It is highly recommended to remove the endotoxin if the DNA is used for endotoxin-sensitive cell lines, primary cultured cells or microinjection. Note: The first elution normally yields 60-80% of the DNA bound. Two elution increases the DNA recovery up to 90%.*

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), -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 vortexing 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. Recentrifuge or vacuum again if necessary.

155 S. Milpitas Blvd., Milpitas, CA 95035 USA | T: (408)493-1800 F: (408)493-1801 | www.biovision.com | tech@biovision.com