Plasmid Midiprep Kit II

(Catalog # K1315-2, -10, -25; Store at multiple temperatures)

I. Introduction:

BioVision's Plasmid Midiprep Kit II is designed for fast and efficient purification of plasmid DNA from 50-100 mL of *E. coli* culture in less than 60 min. The midi column has a plasmid DNA binding capacity of 500 µg. 200-400 µg of high copy number plasmid DNA can be isolated from 100 mL culture. The purified DNA is ready for high performance of downstream applications such as transfection of robust cells such as HEK293, restriction mapping, library screening, sequencing, as well as gene therapy and genetic vaccinations. Unlike other kits in the markets, no chaotropic salts are contained in the buffer of our patented plasmid purification kit. The purified DNA is guanidine/anion exchange resin residues free.

II. Sample Type:

For fast and efficient purification of plasmid DNA from 50-100 mL of *E. coli* culture.

III. Kit Contents:

	K1315-2	K1315-10	K1315-25	
Components	**2 preparations**	**10 preparations**	**25 preparations**	**Part Number**
ezBind Columns	2	10	25	K1315-XX-1
Buffer A1	11 mL	55 mL	135 mL	K1315-XX-2
Buffer B1	11 mL	55 mL	135 mL	K1315-XX-3
Buffer C1	14 mL	70 mL	170 mL	K1315-XX-4
Buffer KB	9 mL	50 mL	110 mL	K1315-XX-5
RNase A (20 mg/mL)	1.1 mg (55 µL)	5.5 mg (275 µL)	13.5 mg (675 µL)	K1315-XX-6
Elution Buffer	4 mL	20 mL	60 mL	K1315-XX-7

IV. User Supplied Reagents and Equipment:

- 70% ethanol and 100% ethanol.
- High speed centrifuge.
- 30 mL high speed centrifuge tubes.
- 50 mL conical tubes.
- 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 room temperature. 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 50-80 mL LB containing appropriate antibiotic with 100 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 6-8 hr with vigorous shaking (~250 rpm). Note: Do not use more than 100 ml culture or cell mass greater than 250. 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 5 mL Buffer A1 (Add RNase A into Buffer A1 before use) and completely resuspend bacterial pellet by vortexing or pipetting (Complete resuspension is critical for optimal yields).
4. Add 5 mL Buffer B1, mix gently but thoroughly by inverting 10 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 6 mL Buffer C1, mix immediately by inverting 5 times and vortex for 10 sec. *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. Transfer the lysate to a high speed centrifuge tube and centrifuge at 14,000g for 10 min at room temperature. *Note: Syringe filter could be used to filtrate the lysate if high-speed centrifuge is not available. Note: If the rotor is cold, put it at room temperature for 10 min and then perform centrifugation as described.*

7. Carefully transfer the clear supernatant into a 50 mL tube (avoid the floating precipitates). Add 6 mL 100% ethanol. Mix immediately by sharp shaking. The mixture of ethanol/lysate needs to be transferred to the DNA column immediately.
8. Immediately transfer the lysate/ethanol mix into a DNA column with the collection tube. Centrifuge at > 2,500g for 1 min at room temperature. Remove the column from the tube and discard the flow-through liquid. Reinsert the column to the collection tube. Repeat step 8 till all the lysate/ethanol mix has been passed through the column.
9. **Optional:** Add 4.0 mL Buffer KB into the spin column, centrifuge at > 2,500g for 1 min. 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.
10. Add 5 mL 70% ethanol into the column, centrifuge at > 2,500g for 1 min. Remove the column from the tube and discard the flow through. Reinsert the column into the collection tube. Repeat step "10".
11. Centrifuge the column, with the lid open, at > 2,500g for 10 min to remove the ethanol residues. *Note: Residual ethanol can be removed more efficiently with the column lid open. It is critical to remove residual ethanol completely.*
12. Carefully transfer the spin column to a sterile clean tube and add 0.5-1 mL ddH_2O or Elution Buffer to the center of the column and incubate for 1 minute at room temperature. Elute the DNA by centrifugation at > 2,500g for 5 min.
13. For higher yield, reload the elute in the 15 mL tube to the column and incubate for 1 min. Elute the DNA again by centrifugation at > 2,500g for 5 min. Note: If ddH_2O is used for elution, make sure that the pH is between 7.0 and 8.5. pH lower than 7 leads to lower elution efficiency. *Note: Two elution give rise to maximum DNA yield. For maximum yield and higher concentration, pool the elution together, add 0.1 volume 3M KAc or NaAc (pH 5.2) and 0.7 volume isopropanol. Mix well and aliquot the sample to 2.0 ml microtubes. Centrifuge at top speed for 10 min. Remove the supernatant. Wash the DNA with 800 µL 70% ethanol, centrifuge for 5 min, carefully decant. Air-dry the pellet for 5-10 min. Resuspend the DNA in Elution Buffer or sterile ddH_2O.*

DNA concentration (µg/ml) = $OD_{260\ nm}$ X 50 X dilution factor

VIII. Purification of Low-Copy-Number Plasmid/Cosmid: The yield of low copy number plasmid is normally around 0.1-1 µg /mL of overnight culture. For isolating low copy number or medium copy number plasmid DNA, use the following guideline:

1. Culture volume: Use 2 X volumes of the high copy number culture.
2. Use 2 X volumes of the Buffer A1, Buffer B1 and Buffer N1.
3. Use same volume of Wash Buffer (70% Ethanol) and Elution Buffer.

IX. Related Products:

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

96-well Plasmid ezFilter Mini Kit	K1325-100 (1 Pack)
96-well Plasmid ezFilter Mini Kit	K1325-100 (4 Packs)

X. 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. Recentrifuge or vacuum again if necessary.

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