Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Express Plasmid Midiprep Kit (25 min)

(Catalog # K1323-2, -10, -25; Store at RT)

I. Introduction:

BioVision's Express Plasmid Midiprep Kit is designed for fast and efficient purification of plasmid DNA from 50-80 mL of E. coli culture. The column has a plasmid DNA binding capacity of 1000 µg. 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 50-80 mL of *E. coli* culture.

III. Kit Contents:

Components	K1323-2 2 preparations	K1323-10 10 preparations	K1323-25 25 preparations	Part Number
ezBind Columns	2	10	25	K1323-XX-1
Syringe Filters	2	10	25	K1323-XX-2
Collection Tubes	4	20	50	K1323-XX-3
Plastic Wrench	1	1	1	K1323-XX-4
Buffer A1	12 mL	60 mL	130 mL	K1323-XX-5
Buffer B1	12 mL	60 mL	130 mL	K1323-XX-6
Buffer C1	15 mL	80 mL	170 mL	K1323-XX-7
DNA Washing Buffer*	5 mL	50 mL	2 x 50 mL	K1323-XX-8
RNase A	50 µL	200 µL	420 µL	K1323-XX-9
Elution Buffer	5 mL	15 mL	30 mL	K1323-XX-10

Add 20 ml (K1323-2) and 200 ml (K1323-10, K1323-25) 100% ethanol to each DNA Wash Buffer before use.

IV. User Supplied Reagents and Equipment:

- 100% ethanol
- High speed centrifuge
- 50mL high speed centrifuge tubes
- 50mL conical 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: 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.
- 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.

VII. 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 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). 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. Attention: Buffer B1 forms precipitation below room temperature, if solution becomes cloudy, warm up at 37°C 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.

VIII. 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)

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.

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