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

Express Plasmid Maxiprep Kit (25 min)

(Cat# K1324-2, -10, -25; Shipping: RT; Storage: Multiple Temperatures)

I. Introduction:

BioVision's Express Plasmid Maxiprep Kit is designed for fast and efficient purification of plasmid DNA from 150-200 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. Key to the kit is our proprietary DNA binding systems that allow the high efficient binding of DNA to our ezBind™ matrix while proteins and other contaminates are removed under certain optimal conditions. Nucleic acids are easily eluted with sterile water or TE buffer. Unlike all other rivals, our patented plasmid purification kit has no guanidine salt in the buffer, the purified DNA is guanidine/ion exchange resin residues free which enable the high performance of downstream applications such as transfection, 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 100-200 mL of *E. coli* culture.

III. Kit Contents:

	K1324-2	K1324-10	K1324-25	
Components	**2 preparations**	**10 preparations**	**25 preparations**	**Part Number**
ezBind Columns	2	10	25	K1324-XX-1
Filter Syringe	2	10	25	K1324-XX-2
Collection Tubes	4	20	50	K1324-XX-3
Plastic Wrench	1	1	1	K1324-XX-4
Buffer A1	22 mL	110 mL	270 mL	K1324-XX-5
Buffer B1	22 mL	110 mL	270 mL	K1324-XX-6
Buffer C1	27 mL	135 mL	340 mL	K1324-XX-7
DNA Washing Buffer*	15 mL	45 mL	2 X 45 mL	K1324-XX-8
RNase A (20 mg/mL)	100 µL	420 µL	900 µL	K1324-XX-9
Elution Buffer	5 mL	15 mL	50 mL	K1324-XX-10

Add 48 ml (K1324-2), 200 mL (K1324-10, -25) of 100% ethanol to each DNA Wash Buffer bottle 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 (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!

VI. Reagent Preparation and Storage Conditions:

- 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.
- 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 RT.

VII. Express Plasmid Maxiprep 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 **150-200 mL LB** containing appropriate antibiotic with 100 µL fresh starter culture. Grow at 37oC for 14-16 h 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 h with vigorous shaking (~250 rpm). Warning: Do not use more than 200 ml culture. Need to scale up buffers if processing more than 200 mL culture.*
2. Harvest the bacterial 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. *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. *Note: Complete resuspension is critical for optimal yield.*
4. Add **10 mL Buffer B1**, mix thoroughly by inverting 10 times with slightly 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. *Attention: Buffer B1 forms precipitation below room temperature, if solution becomes cloudy, warm up at 37oC 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:

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.

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 feel resistance, some of the lysate may remain in the flocculent precipitate. Note: To avoid clog 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 for 5 times. Proceed with plunger protocol described below or vacuum manifold protocol on Page 8.
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 screw the end component back to the maxi column tightly. 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 to the 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.
14. 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. *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.*

Vacuum manifold method (From step 7):

1. Pull out the plunger of the DNA column and insert the column to the manifold with the vacuum off. Transfer the lysate/ethanol mix to the column and turn on the vacuum till all sample passes 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 column 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 to the tube. Spin the column at max speed for 2 min. Transfer the column 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.

VIII. 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.