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

ToxOut™ Endofree Express Plasmid Midi Kit

(Cat# K1335-2, -10, -25; Store at RT)

I. Introduction:

BioVision's ToxOut™ Endofree Express Plasmid Midi Kit is designed for fast and efficient purification of plasmid DNA from 15-50 mL of *E. coli* culture. The mini column has a DNA binding capacity of 250 µg. The purified endofree DNA is ready for downstream applications such as transfection of endotoxin-sensitive cell lines, primary cultured cells or microinjection. Plasmid isolated with traditional protocol normally contains high level of endotoxins (lipopolysaccharides or LPS). For transfection of endotoxin sensitive cell lines or microinjection, the endotoxins should be removed before the applications. The endofree system uses a specially formulated buffer that extracts the endotoxin from the plasmid DNA. Two rounds of extraction will reduce the endotoxin level to 0.1 EU (Endotoxin) per µg of plasmid DNA. The Toxout™ Endofree Express Plasmid Midi Kit provides an efficient endotoxin removal step into the traditional purification procedure to produce transfection grade plasmid DNA. The yield of plasmid DNA depends on the origin of the replication and the size of the plasmid. 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.

II. Sample Type:

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

III. Kit Contents:

Components	K1335-2 2 Preparations	K1335-10 10 Preparations	K1335-25 25 Preparations	Part Number
ezBind Midi Columns	2	10	25	K1335-XX-1
Syringe Filters	2	10	25	K1335-XX-2
Buffer A1	6 mL	60 mL	130 mL	K1335-XX-3
Buffer B1	6 mL	60 mL	130 mL	K1335-XX-4
Buffer N3	3 mL	30 mL	70 mL	K1335-XX-5
Buffer RET	12 mL	70 mL	260 mL	K1335-XX-6
DNA Wash Buffer*	5 mL	50 mL	2 x 50 mL	K1335-XX-7
RNase A	0.6 mg (30 µL)	210 µL	450 µL	K1335-XX-8
Endofree Elution Buffer	4 mL	15 mL	30 mL	K1335-XX-9
Collection Tubes	4	30	30	K1335-XX-10
Plastic Wrench	1	1	1	K1335-XX-11

**Add 200 mL of 100% ethanol to each DNA Wash Buffer bottle before use. Add the RNase A solution to buffer A1 and mix well before use.*

IV. User Supplied Reagents and Equipment:

- High speed microcentrifuge
- 100% ethanol
- 1.5 mL Eppendorf 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 (22-25°C). 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 half a year under 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.
- Buffer N3 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. Pre-warm Elution Buffer at 60°C before elution.

VII. Endofree Express Midiprep Plunger Protocol:

The yield of plasmid DNA depends on the origin of the replication and the size of the plasmid. 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 80 mL culture. Need to scale up buffers if processing more than 80 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 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 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 2.0 mL Buffer N3, mix completely by inverting the tube 10 times and shaking for 2 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 (14,000-18,000 g) for 15 min at room temperature! Transfer the cleared lysate to a 50 ml conical tube.*
   Filter syringe: Pour the lysate into the barrel of the filter syringe and set the syringe in a 50 mL conical tube. Incubate at room temperature for 10 min. 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 50 mL of overnight culture and mix the lysate well after adding Buffer N3. Alternatively, transfer the lysate to another syringe filter.*
7. Add 6 mL of Buffer RET (1 volume of the clear lysate), and 6 mL of 100% ethanol. Mix well by vortexing for 5 sec. Proceed with centrifugation protocol as described below.
8. Gently pull the plunger out from a DNA column and set the column in a 50 mL conical tube. Add 20 mL lysate/Ethanol mixture into the DNA column. Using the plunger. gently expel the lysate through the column. Gently pull out the plunger and process the remaining lysate/ethanol mix as described. Discard the flow-through.
9. Gently pull the plunger out, add 10 ml DNA Wash Buffer, and expel the Buffer out with the plunger.
10. Use the plastic wrench **(provided in the kit)** to detach the end component from the midiprep column and insert it into a 1.5 ml Eppendorf tube
11. 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 1 min. Transfer the column to a new Eppendorf tube.
12. Add 400 µL Endofree 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 12,000 rpm for 1 min.
13. Add the eluted DNA back to the column for a second elution. The first elution normally yields about 60-70% of the DNA and the second elution yields another 20-30% of the DNA. Not: The DNA is ready for downstream applications such as cloning, RFLP, sequencing, and transfection of Endotoxin sensitive cells.

VIII. Related Products:

Product Name	Cat. No.
ToxOut™ Endofree Plasmid Mini Kit	K1326-50
ToxOut™ Endofree Plasmid Mini Kit	K1326-250
ToxOut™ Endofree Plasmid Mini Kit II	K1327-50
ToxOut™ Endofree Plasmid Mini Kit II	K1327-250
ToxOut™ Endofree Plasmid Midi Kit	K1328-2
ToxOut™ Endofree Plasmid Midi Kit	K1328-10
ToxOut™ Endofree Plasmid Midi Kit	K1328-25
ToxOut™ Endofree ezFilter Plasmid Midi Kit	K1329-2
ToxOut™ Endofree ezFilter Plasmid Midi Kit	K1329-10
ToxOut™ Endofree ezFilter Plasmid Midi Kit	K1329-25
ToxOut™ Endofree Plasmid Maxi Kit	K1330-2
ToxOut™ Endofree Plasmid Maxi Kit	K1330-10
ToxOut™ Endofree Plasmid Maxi Kit	K1330-25
ToxOut™ Endofree ezfilter Plasmid Maxi Kit	K1331-2
ToxOut™ Endofree ezfilter Plasmid Maxi Kit	K1331-10
ToxOut™ Endofree ezfilter Plasmid Maxi Kit	K1331-25
ToxOut™ Endofree Plasmid Mega Kit (3 mg)	K1332-1
ToxOut™ Endofree Plasmid Mega Kit (3 mg)	K1332-2
ToxOut™ Endofree Plasmid Mega Kit (3 mg)	K1332-10
ToxOut™ Endofree Plasmid Mega Kit (6 mg)	K1333-1
ToxOut™ Endofree Plasmid Mega Kit (6 mg)	K1333-2
ToxOut™ Endofree Plasmid Mega Kit (6 mg)	K1333-10
ToxOut™ Endofree Plasmid Mega Kit (10 mg)	K1334-1
ToxOut™ Endofree Plasmid Mega Kit (10 mg)	K1334-2
ToxOut™ Endofree Plasmid Mega Kit (10 mg)	K1334-10
ToxOut™ Express Endofree Plasmid Midi Kit	K1335-2
ToxOut™ Express Endofree Plasmid Midi Kit	K1335-10
ToxOut™ Express Endofree Plasmid Midi Kit	K1335-25
ToxOut™ Express Endofree Plasmid Maxi Kit	K1336-2
ToxOut™ Express Endofree Plasmid Maxi Kit	K1336-10
ToxOut™ Express Endofree Plasmid Maxi Kit	K1336-25

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.
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 again if necessary.

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