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ToxOut™ Endofree Plasmid Midi Kit

(Cat# K1328-2, -10, -25; Store at RT)

I. Introduction:

BioVision's ToxOut™ Endofree 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 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 endotoxin free plasmid DNA from 15-50 mL of *E. coli* culture.

III. Kit Contents:

Components	K1328-2 2 Preps	K1328-10 10 Preps	K1328-25 25 Preps	Part Number
ezBind Columns	2	10	25	K1328-XX-1
Syringe Filters	2	10	25	K1328-XX-2
Buffer A1	6 mL	60 mL	70 mL	K1328-XX-3
Buffer B1	6 mL	60 mL	70 mL	K1328-XX-4
Buffer N3	3 mL	15 mL	30 mL	K1328-XX-5
Buffer RET	12 mL	60 mL	135 mL	K1328-XX-6
DNA Wash Buffer* ***(Add 200 mL Ethanol before use)***	12 mL	50 mL	2 x 50 mL	K1328-XX-7
Endofree Elution Buffer	3 mL	15 mL	40 mL	K1328-XX-8
RNase A	30 µL	200 µL	450 µL	K1328-XX-9

DNA Wash Buffer: Add 200 mL 96%-100% ethanol to each bottle before use.

IV. User Supplied Reagents and Equipment:

- High speed microcentrifuge
- 70% ethanol and 100% ethanol
- 30 mL high speed centrifuge tubes
- 15 mL and 20 mL Conical 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.
- 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.
- **DNA Wash Buffer: Add 200 mL 96%-100% ethanol to each bottle before use.**
- Make sure the availability of centrifuge, especially, after mixing the lysate with ethanol, the sample needs to be processed immediately by centrifugation.
- Carry out all centrifugations at RT.

VII. Endofree Plasmid Midiprep Spin Purification 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 **15-80 mL LB** containing appropriate antibiotic with 50 µL fresh starter culture. Inoculate 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 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 **5.0 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). Then add 125 µL Buffer ER into the suspended bacterial culture. Mix well by inverting 5-10 times.
4. Add **5.0 mL Buffer B1**, mix gently but thoroughly by inverting 5 times and incubate for 5 min to obtain a slightly clear lysate to obtain a slightly clear lysate. Then add 250 µL Buffer D1, mix gently and incubating for another 5 min. *Note: Do not incubate longer than 5 min. Over-incubating causes genomic DNA contamination and plasmid damage. Note: Buffer B1 precipitates (cloudy look) below room temperature. Warm up Buffer B1 at 50°C to dissolve precipitation before use.*

5. Add **1.5 mL Buffer N3**, mix immediately by inverting 5 times and sharp hand shaking for 5 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. Transfer the lysate to a high-speed centrifuge tube and centrifuge at 12,000g for 10 min at room temperature. *Note: Syringe filter could be used to filtrate the lysate if high-speed centrifuge is not available.*
7. Carefully transfer the clear lysate to a clean 15 mL tube (avoid the interface precipitates)
8. Add **5 mL Buffer RET** and 5 mL of 100% ethanol. Mix well by sharp hand shaking for 5 times. The mixture of ethanol/lysate needs to be transfer to the DNA column immediately.
9. Immediately transfer the sample solution to a DNA column with a collection tube. Centrifuge at 5,000g for 5 min at room temperature. Remove the column from the tube and discard the flow-through liquid. Reinsert the column to the collection tube. Repeat till all the lysate/ethanol mix has been passed through the column. *Note: If the 15 mL collection tube doesn't match the rotor (for example, the lid of rotor cannot close), the column with the collection can be centrifuged at a benchtop centrifuge at 3,000 rpm for 5 min. Swing bucket rotor is preferred.*
10. Add **10 mL DNA Wash Buffer** to the column and centrifuge at 5,000g for 5 min at RT. Discard the flow through liquid and put the column back to the column. Centrifuge the column for 15 min at a maximum speed to remove the ethanol residues.
11. Carefully transfer the spin column into a clean 50 mL tube and add **0.5-1.0 mL Endofree Elution Buffer** to the center of the column and incubate for 1 min at room temperature. Elute the DNA by centrifugation at 5,000g for 5 min.
12. Reload the elute into the center of the column and incubate for 1 min. Elute the DNA by centrifugation at 5,000g for 3 min. *Note: The DNA is ready for downstream applications such as cloning/subcloning, RFLP, Library screening, in vitro translation, sequencing, transfection, and microinjection. Note: Two elution give rise to maximum DNA yield.*

VIII. Related Products:

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